INFLUENCE OF HUMIC SUBSTANCE STRUCTURE AND COMPOSITION ON INTERACTIONS WITH HYDROPHOBIC ORGANIC COMPOUNDS

By

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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The influence of the structure and composition of humic substance on interactions with hydrophobic organic compounds was investigated for thirteen different humic substances. The structure and composition of the humic substances were determined using a variety of analytical techniques including elemental analysis, ultraviolet/visible spectroscopy, copper binding capacity, total acidity titration, infrared spectroscopy and ^{13}C nuclear magnetic resonance spectroscopy. The results of these analyses indicated that there were significant variations in the structure and composition of the different humic substances.

Partition coefficients (K_{doc}) were measured for each humic substance with two different homologous series of hydrophobic organic compounds, i.e. polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons

(PAHs). Linear correlations were observed between the aqueous solubility of the hydrophobic organic compounds and the partition coefficients for each humic substance. The PCBs were observed to have higher activity coefficients for each humic substance than did the PAHs. These observations support a partition mechanism for these interactions.

The partition experiments revealed at least one order of magnitude variation in the K_{doc} values for a particular hydrophobic organic compound and the different humic substances. Correlations were found between the $\log(K_{doc})$ values for both the PCBs and PAHs with a number of structural and compositional characteristics of the humic substances. The aromatic carbon and carboxylic acid content of the humic substances appeared to influence interactions with the hydrophobic organic compounds. The K_{doc} values determined for the PCBs were more sensitive to oxygen-containing functional groups than those for the PAHs. Differences among the activities of the PCBs and PAHs toward the humic substances were correlated with the atomic oxygen/carbon ratio and total acidity values.

The results of this investigation support a partition mechanism for the interactions between hydrophobic organic compounds and dissolved humic substances. Both the aromatic carbon and carboxylic acid content are important humic substance structural characteristics in determining the strength of their interactions with hydrophobic organic compounds.

CHAPTER 1
INTRODUCTION

Humic substances are a class of naturally occurring organic compounds which are the products of the biological and chemical degradation of biological substances. Although they are of biological origin, humic substances are highly resistant to biodegradation. The biological oxygen demand (BOD) of water containing dissolved humics has been shown to be essentially zero (Black and Christman, 1963; Malcolm, 1985).

Humic substances are ubiquitous in soil, sediment and water. These complex macromolecules do not consist of a single unique chemical structure and are diverse mixtures of polyfuntional macromolecules which span a large molecular weight range. Due to their complex and varied structure, humic substances have been operationally defined by their solubility in aqueous acid and base (Aiken *et al.*, 1985). These definitions are:

Fulvic Acid: that fraction of humic substances that is soluble under all pH conditions

Humic Acid: that fraction of humic substances that is not soluble at pH 2, but which is soluble at higher pH values

Humin: that fraction of humic substances that is not soluble in water at any pH.

Recently, a new operational definition for aquatic humic substances has been proposed (Clesceri *et al.*, 1989). This definition is based on the interaction of the humic substances with a hydrophobic adsorbent, XAD-8 resin. This adsorbent has been extensively used to concentrate dissolved aquatic humic substances from aqueous samples (Leenheer, 1981; Aiken, 1985).

Despite the lack of knowledge of the specific structure of humic substances, these compounds have been shown to play major roles in soil, sediment and water chemistry as photochemical sensitizing agents, precursors to disinfection by-product formation, ion exchangers, surfactants, chelating agents and sorbents for hydrophobic organic compounds (HOCs).

Interactions of Humic Substances with Hydrophobic Organic Compounds

Soil and Sediment Humic Substances

The operational definition of humic substances has obscured the basis for many of the observed interactions of humic substances with other classes of chemicals found in the environment. Humic substances are known to interact with both heavy metals (Truitt and Weber, 1981b; McKnight *et al.*, 1983) and hydrophobic organic pollutants (Karichkoff,

1981; Thurman, 1985). The role of humic substances in the binding and transport of pollutants has been extensively studied in both the dissolved and condensed phases (Chiou *et al.*, 1979; Karichkoff, 1981; Carter and Suffet, 1982; Stevenson, 1982; Landrum *et al.*, 1984; Whithouse, 1985; Chiou *et al.*, 1986; Morehead *et al.*, 1986; Gauthier *et al.*, 1987; McCarthy *et al.*, 1989; Jota and Hassett; 1991). However, due to the complex nature of humic substances, the actual mechanisms for these interactions have yet to be determined.

Early attempts to empirically describe the sorption of hydrophobic organic compounds from water by soil and sediment humic substances using classical solid/liquid sorption isotherms were quite successful (Chiou *et al.*, 1979; Karichkoff, 1981). The organic carbon content of the soil and the hydrophobicity of the organic compound were found to be the major factors controlling the distribution of the pollutant between the water and soil. Many correlations have been found between the aqueous solubility or octanol/water partition coefficient (K_{ow}) of the hdyfrophobic organic compound (HOC) and the soil/water partition coefficient which has been normalized to the soil organic carbon content (K_{oc}). Means *et al.* (1980) derived a general expression for the sorption of 22 hydrophobic compounds by a variety of soils and sediments:

$$\log K_{oc} = \log K_{ow} - 0.317 \qquad r^2 = 0.980 \qquad (1.1)$$

These results are in agreement with those reported by Chiou *et al.* (1979) who observed a similar relationship for the behavior of 15 hydrophobic compounds using different soils. Although these two groups obtained similar results, their interpretations of the basis for the correlations were very different. Means *et al.* (1980) used a standard adsorption model to explain the interaction, while Chiou *et al.* (1979) proposed a partition model for the same interactions. Adsorption is generally used to describe an interaction between a solute and adsorbent which is based on physical or chemical bonding forces. Partitioning refers to the distribution of a solute between two immiscible or partially miscible phases. Partition behavior is governed primarily by van der Waals forces (Kile and Chiou, 1989).

Dissolved Humic Substances

The interaction of aqueous dissolved humic substances with hydrophobic organic pollutants presents conceptual problems for either a sorption or partition model. If the humic material is truly dissolved, there is no second bulk phase for sorption or partitioning (Carter and Suffet, 1982; Chiou *et al.*, 1986; Chiou *et al.*, 1987). A large number of investigators have found that the presence of dissolved

organic carbon (DOC) increases the apparent water solubility of hydrophobic pollutants. Poirrier *et al.* (1972) reported that the presence of DOC in natural stream water increased the apparent solubility of 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (DDT) fourfold. The presence of dissolved humic substances has been reported to decrease the partitioning of hydrophobic pollutants into sediment, suspended solids and biota (Means *et al.*, 1980; Hassett and Anderson, 1982; Oliver and Nilmi, 1983). The presence of DOC in water has also been shown to decrease the toxicity of pollutants to a variety of aquatic organisms (Eadie *et al.*, 1982; McCarthy, 1983; Landrum *et al.*, 1985; Bitton *et al.*, 1986). Regardless of how these interactions are viewed, information reported in the literature indicates that dissolved humic substances can greatly effect the transport and fate of hydrophobic organic pollutants.

Equilibrium constants for the DOC/hydrophobic organic compound interactions (K_{doc}) have been measured for a variety of natural waters and humic substances extracted from soil and sediments which have been dissolved in water. The magnitude of the interactions for a given HOC with DOC from different sources has been reported to vary considerably. Chiou *et al.* (1987) reported that a soil- derived, commercial humic acid had K_{doc} values for two PCBs which were 4 to 20 times the K_{doc} values measured for natural, aquatic DOC. Other research has shown that the K_{ow} of the HOC

accounts for only 46-50% of the observed variation in the K_{doc} values measured with different sources of dissolved humic substances (Landrum *et al.*, 1987; Evans, 1988). Observations of this type have prompted many investigators to postulate that the variations in K_{doc} for a given HOC with different sources of dissolved humic substances is due to the chemical and structural characteristics of the humic substances (Whitehouse, 1985; Chiou *et al.*, 1986; Morehead *et al.*, 1986; Gauthier *et al.*, 1987; McCarthy *et al.*, 1989; Jota and Hassett; 1991). Although many researchers have come to this conclusion, few have attempted to correlate the structure or chemical composition of humic substances with the variation of the K_{doc} values measured for HOCs.

The Role of Humic Substance Structure and Composition in Interactions with Hydrophobic Organic Compounds

The chemical composition of humic substances has been extensively investigated by geochemists (Stevenson, 1982; Ertel and Hedges, 1983; Steelink *et al.*, 1983; Huffman and Stuber; 1985; MacCarthy and Rice, 1985; Wilson, 1987; Rice and MacCarthy, 1991). Humic materials from both terrestrial and aquatic systems have been characterized by a variety of physical and chemical methods. For example, infrared (IR) and nuclear magnetic resonance (NMR) spectroscopic investigations of the structure and functional group compositon of humic substances have revealed some general differences between humic materials from different sources.

NMR spectroscopy has shown that the aromatic carbon content of humic materials varies from 20 to 75% (Hatcher *et al.*, 1983; Wilson, 1987). Terrestrial humic materials have been shown to be more aromatic in character than marine humic substances by both NMR (Hatcher *et al.*, 1983; Wilson, 1987) and IR (Stevenson, 1982) spectroscopy. Both these techniques indicate that fulvic acids are generally less aromatic than humic acids, but fulvic acids contain more carbohydrate character (Stevenson, 1982; Aiken *et al.*,1985). Other techniques which have been used to investigate the chemical composition of humic substances include elemental analysis (Huffman and Stuber, 1985; Steelink, 1985), total acidity titrations (Schnitzer and Khan, 1972; Stevenson, 1982; Perdue; 1985), metal binding capacity (Steveson *et al.*, 1982; McKnight *et al.*, 1983; Thurman, 1985) and UV/VIS spectroscopy (Stevenson, 1982; Ertel and Hedges, 1983; Gauthier *et al.*, 1987; Traina *et al.*, 1990; Novak *et al.*, 1991).

Since humic substances are mixtures of extremely complex macromolecules, any attempt to determine their structure or chemical composition is difficult. The application of any analytical method to determine the functional group composition, molecular weight or tertiary structure of humic substances is in reality the application of the technique to a mixture. Therefore, the information

gathered in these experiments tends to be net or average responses (MacCarthy and Rice, 1985).

The difficult nature of the investigation of the relationship between the structure and composition of humic substances and the variation in the K_{doc} has generally prevented researchers from gathering the data needed for a full understanding of the mechanism of these interactions. Gauthier *et al.* (1987) has investigated their relationship for pyrene and a number of dissolved humic substances from both terrestrial and marine sources. The humic substances used in this study were characterized by elemental analysis, NMR and IR spectroscopy. They observed a linear correlation ($r^2 = 0.48$) between the K_{doc} for pyrene and the atomic H/C ratio of the humic substances. The K_{doc} was also found to correlate linearly ($r^2 = 0.94$) with the aromatic carbon content (measured by NMR) of four of the humic substances. Since both aromatic and carboxylic acid functional groups have the same atomic H/C ratio, the authors suggested that the carboxylic acid functional groups adversely affected the correlation between the atomic H/C ratio and the K_{doc}. These data support the postulates of many researchers who believe that the variation in K_{doc} for a particular HOC with different sources of humic substances is due to their structure and composition.

Although little data are available on the role of oxygen in the interactions of humic substances and

hydrophobic pollutants, its effect on the polarity of humic compounds is strong (Kile and Chiou, 1989). Garbarini and Lion (1986) have found a correlation between the K_{doc} for toluene and trichloroethene and a mathematical function which includes both the C and O content of the humic substances. The reported decrease of K_{doc} for a particular HOC with a single humic source as the pH is increased also suggests the importance of oxygen-containing carboxylic acid functional groups in these interactions (Kile *et al.*, 1989; Jota and Hassett, 1991).

The work of Gauthier *et al.* (1987) and others (Kile *et al.*, 1989; McCarthy *et al.*, 1989; Jota and Hassett, 1991) indicates that the structure and composition of dissolved humic substances is an important factor in the strength of their interactions with HOCs. However, the data needed to fully understand the mechanism of these interactions are currently not available in the literature (Gauthier *et al.*, 1987; Evans, 1988; Kile and Chiou, 1989; McCarthy 1989). The research described in this dissertation was designed to obtain data which will be used to evaluate the importance of the structure and composition of humic substances in their interactions with hydrophobic organic compounds. The data needed to understand the interactions of humic substances with HOCs can be divided into two areas: (i) characterization of the structure and compostion of the humic substances and (ii) characterization of the HOC/humic

substance interactions (measurement of K_{doc}) for the humic substances used in (i).

Humic substances with a wide range of structure and composition were isolated from soils, sediments and water. A variety of different humic sources were chosen to increase the variation in interactions with HOCs and measured compositional characteristics. The investigation of the structure and functional group composition of humic substances was carried out using the following analytical techniques:

1. Elemental Analysis
2. UV/VIS Spectroscopy
3. Total Acidity Titration
4. Copper Binding Capacity
5. Infrared Spectroscopy
6. Nuclear Magnetic Resonance Spectroscopy

The information obtained from these structural and compositional analyses were used to investigate the role of humic substance structure and composition in determining the mechanism and strength of their interactions with two homologous series of HOCs.

Chapter 2 of this dissertation describes the isolation of thirteen different humic substances from soil, sediment and water. The structural and compositional characterization of these humic substances by the methods discussed above is covered in Chapter 3. The interactions

of the HOCs with the humic substances isolated in this study are detailed in Chapter 4. This Chapter also includes a discussion of the correlations obtained between the $\log(K_{doc})$ values measured for these humic substances and their structure and composition. The implications of the composition and structure of these humic substances for understanding their interactions with hydrophobic organic compounds are summerized in Chapter 5.

CHAPTER 2
COLLECTION AND ISOLATION OF HUMIC SUBSTANCES

Sample Collection

Humic substances with a wide range of compositional and structural variation were needed to perform this research. The rationale for this was that a wide range of humic substance sources would likely exhibit the variation in elemental composition and functionality necessary to investigate the effect of these parameters on the interactions between hydrophobic organic compounds and humic substances. Therefore, a variety of humic substances were isolated from several aqueous, terrestrial and sedimentary environments.

Aqueous samples were obtained from two blackwater rivers in Northcentral and Northeast Florida. Additional aqueous samples were obtained from an eutrophic lake and a surficial groundwater source in a planted pine forest. Soil samples were collected from a planted pine forest and a mixed hardwood forest. Sediment samples were collected from the bottom of a eutrophic lake. The sampling and source information for the humic substances used in this investigation is summarized in Table 2.1.

Table 2.1. Type, location, and habitat from which humic substances were collected for the present study.

NAME	TYPE	LOCATION	HABITAT
Aldrich	humic acid / soil	Aldrich Chemical Co. (Lot no. 121137)	soil
IHSS	humic acid / soil	International Humic Substances Society (IHSS Code 1S102H)	soil
Pine Mountain	soil	Pine Mountain State Park, Harris County, Georgia	mixed hardwood / pine forest
Orange Heights	soil	Orange Heights, Alachua, County, Florida	planted pine forest
Newnans Lake 11[1]	sediment	NW section of Newnans Lake, Alachua County, Florida	eutrophic lake
Newnans Lake 16[1]	sediment	central section of Newnans Lake, Alachua County, Florida	eutrophic lake
Newnans Lake 18[1]	sediment	SW section of Newnans Lake, Alachua County, Florida	eutrophic lake
Santa Fe River	DOC[2] / aquatic	Santa Fe River, SR 241 bridge, Alachua County, Florida	blackwater river
St. Marys River	DOC / aquatic	St. Marys River, SR 2 bridge, Nassau County, Florida	blackwater river
Orange Heights	DOC / aquatic	Orange Heights, Alachua County, Florida	groundwater from planted pine forest

Table 2.1 (continued)

NAME	TYPE	LOCATION	HABITAT
Newnans Lake	DOC / aquatic	Newnans Lake, dam tailwater Alachua County, Florida	eutrophic lake
Suwannee River	fulvic acid / aquatic	Suwannee River dam Clinch County, Georgia (IHSS Code 1S101F)	blackwater river
Suwannee River	humic acid / aquatic	Suwannee River dam Clinch County, Georgia (IHSS Code 1S101H)	blackwater river

[1] Göttgens, (1992)
[2] Dissolved organic carbon

Water samples containing dissolved organic matter (DOM) were obtained at each site using a submersible centrifugal pump (K-VA Analytical Systems, model XP-100 Minipump). The pump was placed approximately 30 cm below the surface of the water and the pump and associated sampling tubing were purged with the source water at a flow rate of approximately 1 L/min for five minutes before the sample was collected. The water was pumped into glass carboys (6 or 12 L) that were transported to the laboratory. The carboys had been previously cleaned in the laboratory with phosphate-free detergent and tap water, and rinsed sequentially with deionized water, dilute HCl and deionized water. The pump and sampling tubing were cleaned after each sampling by purging with deionized water for approximately 15 min. The water samples were transported to the laboratory at ambient temperature and processed as described later.

Soil samples were collected using a stainless steel trowel to obtain approximately 500 g of material at a given site. The soil was placed into a plastic bag and transported to the laboratory on ice. Soil samples were stored at 10° C until they were extracted. Sediment samples were obtained using a Livingstone piston corer. The cores were return to the laboratory, extruded and then sectioned into 1 cm sections (Göttgens, 1992). Three sections covering the range from 16 to 24 cm were combined from each sampling location to form a single sample for extraction of

humic substances. The soil and sediment samples were extracted as described below.

Three additional humic substances were obtained in extracted form from the International Humic Substances Society (Golden, CO). These samples included two aqueous humic substances and a soil humic substance. These samples were isolated as described by Malcolm *et al.* (1989), procedures which were essentially those used for the isolation of similar samples in this research. Aldrich humic acid (Cat. No. H1675-2, Lot No. 121137) was also included as a humic substance; this was known to be derived from a soil source. Aldrich humic acid was included because it has been studied extensively and would allow for ready comparison of literature results with those in this research.

Isolation of Humic Substances

Isolation of aquatic and soil/sediment organic matter from natural matrices was necessary for a number of reasons. Since the objective of this research was to investigate the interactions between hydrophobic organic compounds (HOC) and dissolved humic substances, the extraction of soil/sediment derived humic substances from the solid matrix and subsequent dissolution for experiments with HOC was necessary. The aqueous humic substances were extracted to provide a concentrated source of this type of humic

substance. Once isolated, aqueous and soil/sediment humic sources could be dissolved in water for investigations carried out under conditions of controlled pH and ionic strength. This was an important consideration because the interactions of a given HOC with a particular humic substance has been shown to vary with ionic strength and pH (Carter and Suffet, 1982; Jota and Hassett, 1991). The final extracts for both aqueous and soil/sediment humic substances were freeze dried. Humic substances in this form, when stored in the dark, are quite stable (Aiken, 1985). The procedures used to characterize the structure and functional group composition of the humic substances were performed collectively. Therefore, the ability to store isolated humic substances in a stable form was important.

Aquatic Humic Substances

Aquatic humic substances were isolated from water samples using the procedure described in Standard Method 5510 C (Clesceri *et al.*, 1989). The basic procedure involves acidification to pH 2 of the 0.45 μm filtered water sample, concentration on a macroporous resin, and elution of the aquatic humic substances by backflushing the column with dilute base. This method is an analytical isolation method and was modified for preparative scale isolation by simply scaling up the size of the resin column. The procedure is

similar to the isolation of the hydrophobic acid fraction of dissolved organic carbon from natural waters reported by Leenheer (1981). This method has been used to reproducibly isolate aquatic humic substances from a variety of water sources on a preparative scale.

Materials and methods

Ionic strength measurements were made using a digital conductivity bridge (Fisher Scientific Co., Cat. no. 09-325-360) which was calibrated by measuring a standard 0.001 M KCl solution (Fisher Scientific Co., Cat. no. P212-100). The pH measurements were made using a Fisher Scientific Model 900 pH meter equipped with a combination pH electrode (Fisher Scientific Co., Cat. no. 13-602-108). The pH meter was calibrated before each use with buffer solutions at pH 1, 4 and 7 (Fisher Scientific Co., Cat. nos. SB140-500, SB101-500 and SB107-500, respectively).

Total organic carbon (TOC) measurements were carried out using an Ionics Model 555 Organic Carbon Analyzer. The dissolved CO_2 present in liquid samples to be analyzed for TOC was removed by acidifying the sample with concentrated nitric acid (Fisher Scientific Co., Cat. no. A467) and stripping with a nitrogen purge. This instrument operates by combusting liquid samples over a copper oxide catalyst at 900° C under nitrogen flow (Airco, UHP Grade, 99.9995%) to produce CO_2. The CO_2 produced is detected by an Horiba

Model PIR-2000 Infrared Detector. Potassium acid phthalate (Mallinckrodt, Inc., Cat. no. 6704) solutions were prepared from solid dried primary standards and used to calibrate this instrument in the range of 1.0 to 10.0 mg/L TOC.

Water samples were filtered under 40 psi nitrogen pressure through a 10.1 cm diameter, 0.45 µm pore size nylon filter (Fisher Scientific Co., Cat. no. NO4SP14225) positioned in a flow through filter holder (Fisher Scientific Co., Cat. no. 09-753-17E). Feed water for filtration was provided from a 5 gal stainless steel reservoir. Filtered water was stored in glass carboys and the pH adjusted to 2.0 with concentrated HCl (Fisher Scientific Co., ACS Certified, Cat. no. A144-212).

The Amberlite XAD-8 resin (Rohm and Haas,lot# CG-360, 16-50 mesh size) was obtained from Supelco, Inc. (part# 2-0278). The resin was packed into a glass low pressure liquid chromatography column (Spectrum Medical Industries, Inc., part# 124026). The column was 45 cm long and had a 2.5 cm internal diameter (ID), yielding 4.91 mL/cm bed volume. The column was fitted with an adjustable plunger endcap (Spectrum Medical Industries, Inc., part# 124122). The column was also fitted with a shut-off valve (Spectrum Medical Industries, Inc., part# 124420) at the exit and a combination shut-off valve/metering valve (Spectrum Medical Industries, Inc., part# 124432) at the entrance. Liquid was

run through the XAD-8 column by gravity feed and the flow rate controlled using the metering valve.

The XAD-8 resin is a macroporous methylmethacrylate co-polymer. The resin is contaminated with leachable organic carbon when it is obtained from the manufacturer. Therefore it must be repeatedly extracted to remove this organic carbon before it is used to concentrate aquatic humic substances (Leenheer, 1981; Clesceri *et al.*, 1989). The resin was initially wetted with 0.1 M NaOH (Fisher Scientific Co., Reagent Grade, Cat. no. S320-500) and the fine particle contaminants were decanted. The resin was then stored in 0.1 M NaOH for at least 24 hours. This solution was decanted and the resin was repeatedly washed with deionized (DI) water followed by a final rinse with methanol (Fisher Scientific Co., Optima Grade, Cat. no. A454-4). The resin was sequentially Soxhlet extracted with methanol, hexane (Fisher Scientific Co., Optima Grade, Cat. no. H303-4) and methanol to remove organic contaminants. The glass chromatography column was packed with a methanol/resin slurry and 8 L of DI water pumped through the column to remove the methanol. The column was further rinsed with 8 L of 0.1 M NaOH followed by 8 L of 0.1 M HCl. The column was considered ready for isolation of humic substances from natural waters when the TOC of the 0.1 M HCl eluent was less than 1 mg/L.

The Spectra/Gel Type 50X8 cation exchange resin (Spectrum Medical Industries, Inc., lot# 16599) was obtained from Fisher Scientific, Inc. (Cat. no. 11 187-130). The resin was reported by the manufacturer to have an exchange capacity of 1.7 milliequivalents/mL (meq/mL). The cation exchange resin was purified by Soxhlet extraction with methanol to remove leachable organic carbon (Leenheer, 1981). The resin was slurry packed into a 35 cm long by 2.5 cm ID glass chromatography column (Fisher Scientific Co., Cat no. K420280-0242) fitted with a 300 mL reservoir. The end of the chromatography column was fitted with a scintered glass frit and a stopcock. Flow through the column was under gravity feed and was controlled at approximately 20 mL/min by the stopcock. The column was packed with a methanol/resin slurry to just below the reservoir and a glass wool plug was used to top the resin. This yielded a bed volume of approximately 150 mL. The column was rinsed with 5 L DI water, then 1 L of 2.0 M HCl to convert the resin to the hydrogen ion form. The final preparation of this column was repeated rinsing with DI water (6-8 L) to remove the HCl. The column was considered ready for use when the conductivity of the column effluent was less than 10 µmhos/cm (Leenheer, 1981).

The calculated cation exchange capacity of the 150 mL bed volume, based on the manufacturer's reported capacity for this resin, was 0.255 meq. The capacity of the bed was

determined experimentally by measuring the volume of 0.1 M NaOH required to achieve column breakthrough. The calculated volume of this solution required to exceed the column exchange capacity was 2.55 L. The results obtained in this experiment are illustrated in Figure 2.1. The effluent pH was monitored to determine when NaOH was eluting from the column. The pH of the effluent remained at 5 until 2.60 L of 0.1 M NaOH had passed through the column. The pH of the effluent was 8.0 at 2.65 L and 11 at 2.7 L. This confirmed that the exchange capacity reported by the manufacturer was accurate.

Once the cation exchange column had been used, it was regenerated by eluting 4-5 L DI water to remove excess DOC. The Na^+ ions were displaced from the column by eluting 1 L of 2 M HCl. The excess HCl was eluted with DI water (approximately 2-3 L) until the conductivity of the column effluent was less than 10 μmhos/cm.

Isolation

Once the XAD-8 column had been prepared and conditioned as described, isolation of aquatic humic substances was initiated. The water samples which had been filtered and adjusted to pH 2.0 were placed approximately 1 m above the XAD-8 column inlet. The flow through the column was set to 12-16 mL/min using the column inlet metering valve. This flow rate was 6 to 8 bed volumes/hr, which is significantly

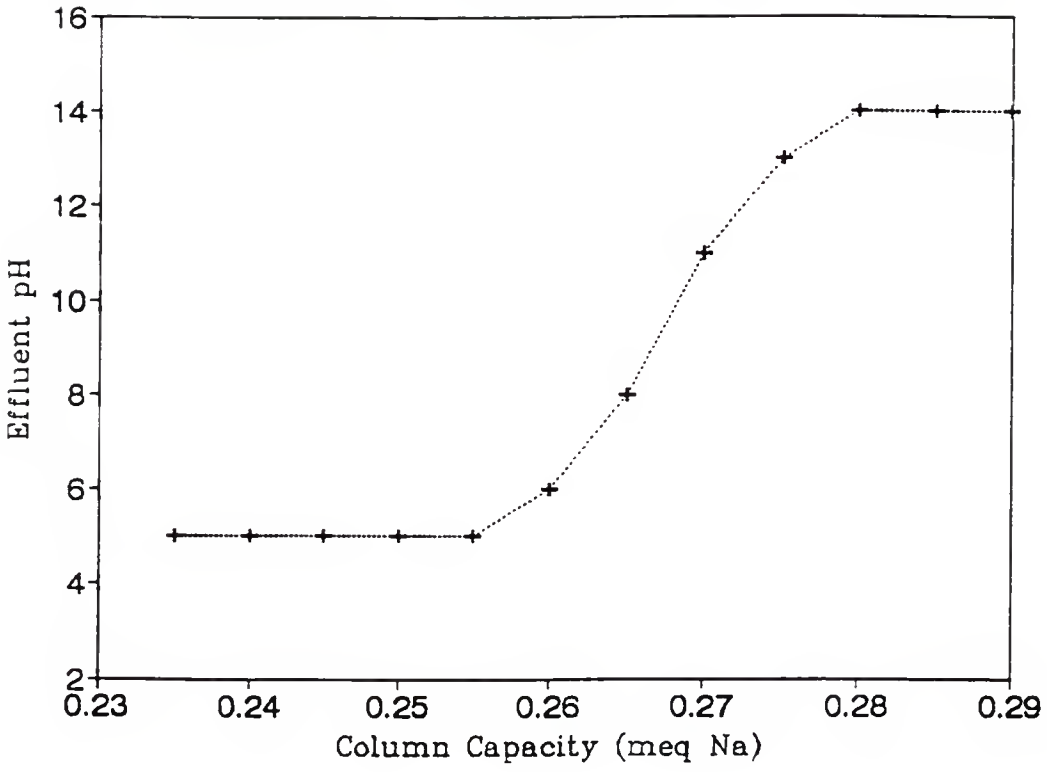

Figure 2.1. Cation exchange column capacity determination.

less than the suggested maximum rate of 30 bed volumes/hr (Leenheer, 1981).

The column effluent total organic carbon (TOC) was used to monitor the breakthrough of both humic and non-humic dissolved organic carbon. The influent TOC was used as the measure of total dissolve organic carbon (DOC) in the sample. The nonhumic DOC fraction was represented by the TOC measured immediately after the XAD-8 column dead volume had been exceeded (75 mL) The humic fraction was considered to be the difference between the total DOC and the non-humic DOC. Based on the TOC measured after the dead volume was eluted, the non-humic DOC fraction generally was about 50% of the total DOC in the water samples investigated. This is in the range reported for this fraction for natural waters by Thurman (1985). The TOC concentration was monitored every 2 L to determine the point at which the capacity of the XAD-8 resin for retaining humic substances had been exceeded. The application of the water sample was stopped once approximately 30% of the humic fraction broke through the column. The application of water was stopped at this point to maximize the total amount of humic substance which could be isolated from a given water source.

The breakthrough of both non-humic and humic DOC is illustrated for the Orange Heights DOC sample in Figure 2.2. In this graph, it can been seen that the non-humic DOC begins eluting from the column almost immediately. The

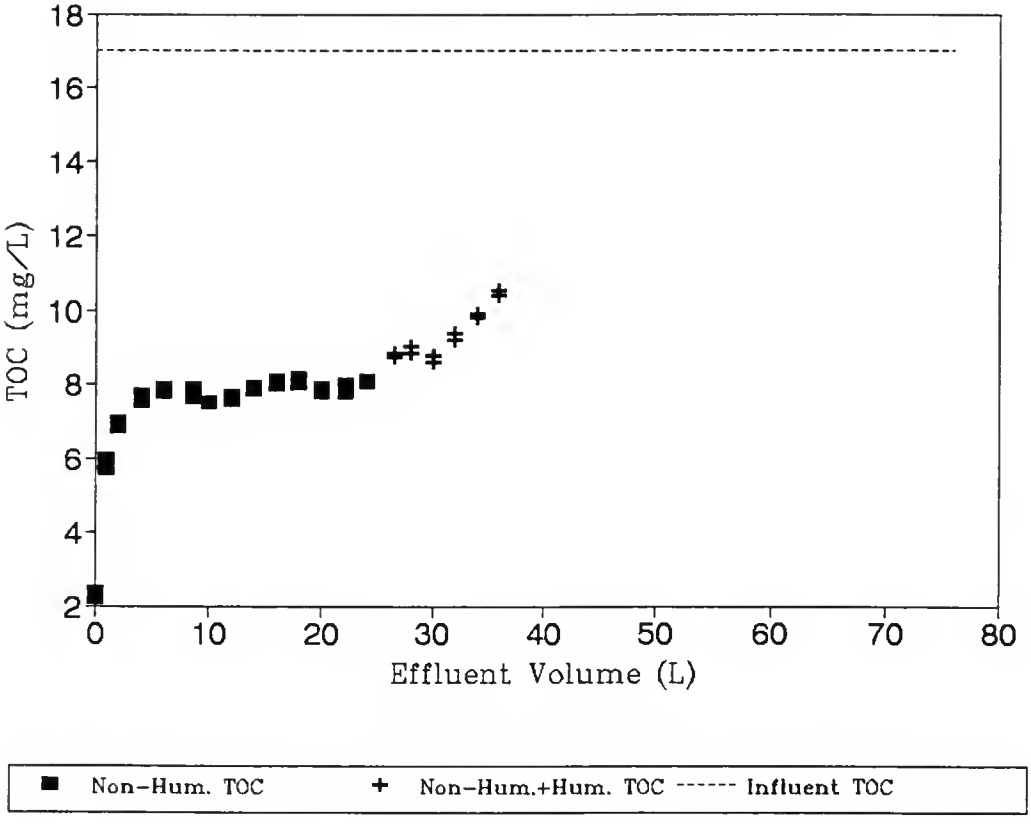

Figure 2.2. XAD-8 column carbon breakthrough determination for Orange Height DOC sample. Note the non-humic TOC breakthrough at 4 L of effluent volume and the non-humic plus humic TOC breakthrough at approximately 26 L. Influent TOC was 17 mg/L and the column void volumn was 75 mL.

non-humic DOC in the column effluent breakthrough occurred within 4 L of the initial elution and maintains a relatively constant level of 7.9 mg/L TOC. The humic DOC began to breakthrough between 24 and 26 L and increased until application of the sample was halted at 36 L (effluent TOC at 10.5 mg/L). Based on the results of this breakthrough curve, the non-humic fraction of the DOC in this sample was 7.9 mg/L (46% of the total DOC concentration of 17 mg/L) and the humic DOC fraction was 9.1 mg/L. The elution was stopped when 2.6 mg/L of humic DOC was breaking through the column (28.5% of the humic DOC). Depending on the total DOC concentration of the water and the fraction of the total DOC which was humic DOC, the effluent volume required to reach the 30% humic DOC breakthrough point was between 24 and 40 L for the water samples investigated.

Aquatic humic substances were eluted from the XAD-8 column by backflushing the column with 0.1 M NaOH. The column was inverted and the 0.1 M NaOH flow rate was set to 1-2 mL/min using the column metering valve. Elution of the humic substances was considered complete when no color was visible in the column effluent. A volume of 125 to 300 mL of NaOH was required to elute the aquatic humic substances from the column. This concentrate was stored in the dark in a 250 mL glass erlenmeyer until it was eluted through the cation exchange column.

The 0.1 M NaOH solutions of concentrated aquatic humic substances were eluted through the cation exchange column which had been prepared as described earlier to convert the humic substances to the H^+ form. The solutions were eluted at a rate of approximately 1-2 mL/min. This eluate was frozen at -40°C and freeze dried to provide the aquatic humic substances in dried form. The dried humic substances were stored in 40 mL glass vials with Teflon lined caps (Fisher Scientific Co., Cat. no. 03-339-18A). These samples were stored at room temperature in a desiccator in the dark until needed.

Soil/Sediment Humic Substances

Soil and sediment humic substances were extracted from the solid matrices using 0.5 M NaOH. Aqueous solutions of NaOH have been the solvents most often used for extraction of soil humic substances for approximately two centuries (Hayes, 1985). Although some alteration of the humic substances extracted with dilute NaOH does occur (Aiken, 1988) NaOH is still the most widely used solvent for extraction of humic substances from soils (Schnitzer and Khan, 1978). The predominant reaction occurring in basic solution has been reported to be ester hydrolysis (Gregor and Powell, 1987; Aiken, 1988). The minor alteration of extracted humic substances is unavoidable and not of great concern in this investigation because all sources of humic

substances were characterized for composition and structure after extraction and isolation procedures were complete.

The humic substances were extracted from soil or sediment by placing the soil/sediment in 0.5 M NaOH at a 1/3 ratio (weight g/volume mL). The mixture was stirred thoroughly and allowed to stand overnight. The supernatant was decanted into 250 mL polycarbonate centrifuge tubes (Fisher Scientific Co., Cat. no. 05-579) and centrifuged at 7500 rpm for 1.5 hrs. After centrifugation, the supernatant was removed from the pellet by pipet and filtered through a 47 mm diameter, 0.45 μm pore size nylon filter (Fisher Scientific Co., Cat. no. NO4SP04700). Soil/sediment humic substance extracts were stored in glass, in the dark, until they were processed through the cation exchange column.

The soil/sediment extracts were converted to the H^+ form by eluting the NaOH extracts through the cation exchange column in the same manner as that described for the aquatic humic substance concentrates. It should be noted that the XAD-8 column eluate concentrates of aquatic humic substance contained only the Na^+ ions present in the 0.1 M NaOH solution. However, the soil/sediment extracts could also contain co-extracted metal ions. The capacity of the cation exchange resin was chosen to insure that it exceeded the capacity needed to remove the Na^+ ions present in the 0.5 M NaOH extracts by a factor of at least four (this assumes 125 mL of 0.5 M NaOH). This leaves sufficient

cation exchange capacity to remove divalent cations from the extract solution up to a concentration equivalent to 200 mg/kg in the original soil.

The soil/sediment humic substance extracts obtained from the cation exchange procedure were frozen at -40°C and freeze dried to obtain the humic substances in dried form. These humic substances were stored in 40 mL glass vials with Teflon lined caps and the vials were stored in a desiccator, in the dark, until analyzed further.

Discussion

Humic substances are operationally defined by the procedures used to extract them from their natural environment (Aiken, 1988). This has been the case since the initial work was performed on the colored extracts of soils. Even the "dissolved" designation of aqueous humic substances is operationally defined as that portion of aqueous humic substances that is not retained by a 0.45 µm filter (Clesceri *et al.*, 1989). Many different methods have been used to isolate humic substances for water (Aiken, 1985). This has lead to difficulty in directly comparing results from studies using different isolation techniques. However, the recently proposed method for isolation and quantitation of aquatic humic substances in Standard Methods (Clesceri *et al.*, 1989) appears to be a move toward a more uniform operational definition of these compounds. This method is

the result of extensive research over the past 20 years on the use of macroporous resins for the isolation of humic substances.

The isolation and concentration of the low levels of naturally occurring dissolved humic substances is generally necessary in any study which includes chemical and/or structural investigation of aquatic humic substances (Aiken *et al.*, 1979; Thurman and Malcolm, 1981). A number of different adsorbents have been used to isolate humic substances from natural waters. These include a number of different synthetic styrene divinylbenzene and acrylic ester polymer resins as well as activated carbon (Aiken *et al.*, 1979; Aiken, 1985; Aiken, 1988) and diethylaminoethyl cellulose (Clesceri *et al.*, 1989; Miles *et al.*, 1983).

The use of activated carbon for the isolation of humic substances from water is simple and inexpensive. However, many authors have noted irreversible sorption of some organic compounds onto the activated carbon (Aiken, 1985). Slow sorption kinetics have also been noted which often limit flow rate. Also, chemical alteration of the isolated organic matter has been reported (Aiken, 1985).

These problems have led many investigators to seek other sorbents for the isolation of aquatic humic substances. Macroporous non-ionic polymer resins have a number of advantages over activated carbon for the isolation of aquatic humic substances. These resins are

also simple, but they have higher capacities for humic substances than activated carbon. Even though these resins are more expensive than activated carbon, they can be easily regenerated and reused. The sorbed humic substances are easily eluted once concentration is complete. The main disadvantage of these macroporous resins is the bleed of organic material from the column material. This problem can be minimized by thorough preparation of the column material before isolation is initiated (Leenheer, 1981).

A number of different macroporous resins have been used to isolate aquatic humic substances. These include both styrene divinylbenzene polymers (XAD-1, XAD-2 and XAD-4) and acrylic ester polymers (XAD-7 and XAD-8). The acrylic ester resins have been shown to have faster sorption kinetics, higher capacities and more efficient elution of sorbed aquatic humic substances than the styrene divinylbenzene resins (Aiken *et al.*, 1979). These advantages have resulted in the adoption of this resin as the sorbent in Standard Method 5510 C (Clesceri *et al.*, 1989). for the isolation and quantitation of aquatic humic substances. The XAD-8 column adsorption procedure has also been selected by the International Humic Substances Society as the isolation method used for the their standard and reference aquatic humic substances.

The procedures used for the isolation and concentration of aquatic humic substances in this research were chosen to

allow the most direct comparison with results available in the literature. Although some alteration of the aquatic humic substances, such as ester hydrolysis (Aiken, 1988), has been shown to occur in the presence of NaOH (used to elute the AHS from the column). This problem was minimized by running the basic XAD-8 column eluates through the cation exchange column and freeze drying these concentrates (Aiken, 1985).

Once the extracts have been freeze dried and placed in the dark, they can be stored until needed without chemical degradation (Aiken, 1985). This is important for the research conducted in this investigation. Since the main objective was to compare the humic substances obtained for different sources in a variety of analytical procedures, it is imperative that the humic substance isolates be stable.

Isolation of humic substances from soil and sediment has received much attention (Stevenson, 1972; Schnitzer and Khan, 1978; Hayes, 1985). Many organic and inorganic solvents systems have been used to extract organic matter from soils. Hayes (1985) compared the humic substances extracted from soils using thirteen different solvent systems. These included dipolar aprotic organic solvents as well as aqueous buffer solutions and NaOH. Hayes (1985) noted slightly higher extraction efficiencies for 2.5 M ethylenediamine (EDA) than for 0.5 M NaOH. However, elemental analysis revealed an increased nitrogen content of

the EDA extracts, indicating incorporation of the amine into the humic substances. A similar result was obtained with dimethylformamide (DMF). Dimethylsulfoxide (DMSO) was found to extract similar levels of organic matter from soils compared to 0.1 and 0.5 M NaOH. The problem with this extraction procedure was that the recovery of the "classic" humic fraction from the DMSO extract required the addition of NaOH, resulting in the same degradation reactions that the organic solvent was designed to prevent. The solvent of choice for the extraction of humic substances from soils and sediments still appears to be aqueous NaOH.

The extracts obtained from the soil and sediment samples in this investigation were passed through a cation exchange column to remove the Na^+ ions and produce the H^+ form of the humic substances. This procedure also removed the OH^- ion by converting it to H_2O. Thus, once the soil/sediment extracts had been passed through the cation exchange resin, the hydrolysis of esters was prevented. As noted for the aquatic humic substances, once the soil humic substances were frozen and freeze dried they were not subject to further degradation (Aiken, 1985).

The aquatic, soil and sediment humic substances were stored in the dark in sealed glass vials in a desiccator until needed for the different characterization experiments described in Chapter 3. The isolation of different types of humic substances to yield solid substances which could be

dissolved in aqueous solution under controlled pH and ionic strength conditions was important for direct comparison of experimental results among these humic sources. The control of these two parameters is significant, considering the complex nature of the humic substances themselves.

CHAPTER 3

CHARACTERIZATION OF HUMIC SUBSTANCE STRUCTURE AND COMPOSITION

The current understanding of the factors that control the association of hydrophobic organic compounds (HOC) with natural dissolved organic carbon (DOC) is inadequate. The association of different HOC with a particular DHS increases as the water solubility of the organic compound decreases (Carter and Suffet, 1983; Chiou *et al.*, 1987; Lara and Ernst, 1989). However, the amount of a given HOC that associates with different DOCs has been shown to vary significantly among different sources of DOC (Chiou *et al.*, 1987; Gauthier *et al.*, 1987; Evans, 1988; McCarthy *et al.*, 1989). Sorption of HOC to soils and sediments has been shown to be controlled by the sorbent organic carbon content (Chiou *et al.*, 1979 ; Karickhoff *et al.*, 1979) . Since less than 50% of the variation in the sorption of HOC by DOC has been attributed to the DOC organic carbon content it has been postulated that structure and composition of DHS is the cause for the observed variation in HOC sorption (Chiou *et al.*, 1987; Gauthier *et al.*, 1987).

The fact that humic substances are complex mixtures of macromolecules has impeded studies designed to investigate

the mechanisms of these interactions. To elucidate the attributes of DOC that affect their sorption of HOC, humic substances obtained from different sources (Table 2.1) were characterized by a variety of physical and chemical techniques. The elemental composition data reported by a number of investigators indicate that humic substances from different sources vary in composition (Huffman and Stuber, 1985; Malcolm and MacCarthy, 1986; Rice and MacCarthy, 1991).

The elemental composition of the humic substances was determined to investigate whether the elemental composition of humic substances could be used to predict their association with HOC. Oxygen-containing functional groups are abundant structural components of humic substances (Schnitzer and Khan, 1972; Stevenson, 1982). The definitions of humic and fulvic acids are based on aqueous solubility that is related to their oxygen-containing functional groups (Thurman, 1985). Therefore, oxygen was expected to play an important role in the interactions between DHS and HOC. The total acid functional group content and copper ion binding capacities were measured as overall estimates of humic substance oxygen-containing functional groups.

Ultraviolet/visible (UV/Vis) spectroscopy has been used extensively by soil scientistS for the investigation of humic substances (Thurman, 1985). The absorbance at 272 nm

for DHS has been correlated with the aromatic carbon content (Traina *et al.*, 1990). The ratio of absorbance at 465nm/665nm (E4/E6) has been correlated with the aromatic carbon content and molecular weight of DHS (Chen *et al.*, 1977). The UV/Vis spectra of the humic substances were investigated because of these relationships and because UV/Vis spectrosocpy is a readily available analytical tool.

Fourier transform infrared (FTIR) and ^{13}C nuclear magnetic resonance (NMR) spectroscopic techniques were employed to determine the functional group composition of the different humic substances. FTIR and ^{13}C NMR yield similar information but are also somewhat complementary (Inbar *et al.*, 1989). The powerful combination of FTIR and ^{13}C NMR for the investigation of the structure as well as the composition of these complex systems has been reported (Lobartini and Tan, 1988; Inbar *et al.*, 1989; Lobartini *et al.*, 1991).

A description of the methods used to characterize the composition and structure of the humic substances is provided in this chapter. The results of each characterization are included as is a discussion of the results relative to the structure and composition of the humic substances investigated.

Elemental Analysis

The elemental composition of a chemical substance can be used to derive the empirical formula and assist in determining a molecular structure. However, with non-stoichiometic materials like humic substances, the usefulness of empirical formulas is limited (Rice and MacCarthy, 1991). Nevertheless, elemental analysis is probably the most widely used method to characterize humic substances (Huffman and Stuber, 1985). Recent reviews of the literature indicate that, even though the data obtained for elemental analysis of humic substances represent averages, there are significant differences among the elemental composition of humic substances from different sources (Steelink, 1985; Rice and MacCarthy, 1991).

The most commonly used method for the determination of C,H, and N is the catalytic combustion of the sample in an oxidizing atmosphere followed by separation and quantification of the resulting CO_2, H_2O and N_2. The oxygen content is most often determined by difference from 100 % (after correction for moisture and ash content), based on the measured quantities of the other three elements (Rice and MacCarthy, 1991). The calculation of oxygen content based on the C,H and N composition assumes that there are no other elements present in humic substances. Although this assumption can present difficulties, it is reasonable (Schnitzer and Khan, 1972; Thurman, 1985) and will be

discussed later in detail. The above method was used to determine the elemental analysis of humic substances investigated in this study.

A potential problem encountered in the determination of oxygen, as well as hydrogen, is moisture. The presence of moisture in samples leads to incorrectly high hydrogen and oxygen values. The most reliable method to avert this problem is to determine the moisture content of the samples used for elemental analysis and to correct the resulting data for the contributions of moisture (Huffman and Stuber, 1985). Another important consideration in the accurate determination of the elemental composition of humic substances is ash content. The ash content of an isolated humic substance is considered a measure of the total inorganic content of the sample. The procedure for measuring ash content is straightforward. The humic substance is placed in a crucible and the organic fraction is oxidized at high temperature (750° C), leaving an ash which represents the inorganic residue.

Methods

Elemental analyses were carried out using a Carlo Erba Model 1106 CHN analyzer. Freeze dried humic substance samples (approximately 2 mg each) were weighed into tin boats and combusted in the presence of oxygen gas over silver cobaltus cobaltus oxide at 1800° to 1850° C. The

resulting combustion gases were separated using a 2 m long, 0.25 inch internal diameter column containing 80 mesh PoraPak QS with helium carrier gas. The analyte gases were detected and quantified employing a thermal conductivity detector that had been previously calibrated with organic compounds of known C, H, and N composition. In this procedure, every fifth sample was a quality control check sample. The elemental composition of all samples of humic substances were analyzed in duplicate. The results of these analyses are presented in Table 3.1.

The moisture content of the humic substances investigated was determined by measuring weight loss on drying, a common method used by researchers in the field (Schnitzer and Khan, 1972; Huffman and Stuber, 1985). Humic substances (approximately 10 mg) were weighed into 4 mL borosilicate glass vials (Fisher Scientific Co., Cat. no. 03-339-25B) and placed in a desiccator. Samples were dried in a vacuum oven (Thelco Model 19, Precision Sci. Co.) at 60° C that was evacuated to 11 mm Hg. The vacuum was released through a $CaSO_4$ drying tube after 24 hr. Samples were transferred to a desiccator, allowed to cool to room temperature and weighed. Weight measurements were carried out using a Mettler Model HK60 balance. The drying procedure was repeated and the samples were reweighed. No difference was noted between the first and second drying. Therefore, a single 24 hour drying under these conditions

Table 3.1. Elemental analysis and composition of humic substances used in the present study and reported in the literature. Moisture, ash, and elemental analyses are expressed as percent by weight. Elemental composition, expressed in moles, appears in parentheses. Atomic ratios are based on elemental composition.

Humic Source	Moisture	Ash [2]	Elements [1] C	H	N	O [3]	H/C	H/O	O/C
Aldrich Humic Acid	2.06	1.50	50.83 (4.24)	3.57 (3.57)	0.60 (0.04)	45.00 (2.81)	0.84	1.27	0.66
IHSS Humic Acid	nd	0.90	57.47 (4.79)	3.75 (3.75)	4.14 (0.30)	33.80 (2.11)	0.78	1.78	0.44
Pine Mt. Soil	1.59	0.20	58.81 (4.90)	5.25 (5.25)	0.48 (0.03)	35.46 (2.22)	1.07	2.37	0.45
Orange Hts. Soil	2.60	12.60	47.67 (3.97)	4.41 (4.41)	0.74 (0.05)	47.19 (2.95)	1.11	1.49	0.74
Newnans Lake Sediment 11	1.38	17.25	46.03 (3.84)	4.80 (4.80)	2.95 (0.21)	46.21 (2.89)	1.25	1.66	0.75
Newnans Lake Sediment 16	5.95	26.95	40.55 (3.38)	5.19 (5.19)	2.20 (0.16)	52.06 (3.25)	1.54	1.59	0.96
Newnans Lake Sediment 18	0.81	15.60	42.34 (3.53)	4.76 (4.76)	2.51 (0.18)	50.39 (3.15)	1.35	1.51	0.89
Santa Fe River DOC	1.65	1.15	49.41 (4.12)	3.69 (3.69)	0.77 (0.06)	46.13 (2.88)	0.90	1.28	0.70

Table 3.1. (continued)

Humic Source	Moisture	Ash	Elements C	H	N	O	H/C	H/O	O/C
St. Marys River DOC	0.62	0.43	51.17 (4.26)	3.82 (3.82)	0.61 (0.04)	44.41 (2.78)	0.90	1.38	0.65
Orange Hts. DOC	1.20	2.35	50.48 (4.21)	4.10 (4.10)	0.49 (0.04)	44.93 (2.81)	0.97	1.46	0.67
Newnans Lake DOC	0.28	6.62	50.23 (4.19)	4.60 (4.60)	0.79 (0.06)	44.38 (2.77)	1.10	1.66	0.66
Suwannee River Fulvic Acid	nd	0.82	53.75 (4.48)	4.29 (4.29)	0.68 (0.05)	40.98 (2.56)	0.96	1.67	0.57
Suwannee River Humic Acid	nd	3.19	54.22 (4.52)	4.14 (4.14)	1.21 (0.09)	39.82 (2.49)	0.92	1.66	0.55
Fulvic Acids [4]			35.4-57.0	2.9-6.9	0.0-5.7	34.6-56.6	0.59-1.83		0.43-1.10
Humic Acids [4]			45.1-65.1	2.8-7.2	0.5-6.5	24.4-47.2	0.85-1.35		0.24-0.76

[1] Elemental analyses, composition, and atomic ratios are corrected for moisture and ash content
[2] Represents approximate inorganic content
[3] Oxygen content calculated by difference
[4] 95% confidence interval from Rice and MacCarthy (1991)
nd: not determined

was considered sufficient for moisture determination. The conditions employed for moisture determination were those suggested by Huffman and Stuber (1985) who have conducted an extensive investigation of the factors effecting moisture determinations of humic substances. They found that weight loss on drying at 60° C corresponded closely with the moisture determined by Karl Fischer titration.

The ash content of the humic substances investigated was determined by measuring the weight lost after ignition at 750° C for two hours. The Mettler Model HK60 balance was also used for the ash determination weighings. Before samples were ashed, the 4 mL covered ceramic crucibles (Coors, Fisher Scientific Co., Cat. nos. 07-965B, porcelain crucible; 07-970C, cover) used in this procedure were checked for constant tare weight by repeated heating to 750° C and cooling in a desiccator. This procedure confirmed that the weights of the crucibles were constant and unaffected by repeated heating to 750° C.

The humic substances were weighed (approximately 10 mg) into tared crucibles. The crucibles were placed in a cool muffle furnace which was then heated to 750° C and the samples were ignited for 2 hours. The furnace was then cooled to near 100° C and the crucibles were removed and placed in a desiccator. This insured that the ash did not become saturated with water from the air. Once the samples reached room temperature, they were weighed. The process

was then repeated. The second heating showed no additional weight loss, indicating that the first heating was sufficient for ash determination.

The moisture and ash content of each humic substance were determined in duplicate. The averages for each of these determinations are included in Table 3.1. The elemental compositions in Table 3.1 have been corrected for moisture and ash content. The oxygen content was determined by difference (from 100%) based on the C,H and N content, after these values had been corrected for moisture and ash.

Results

The most reliable information obtained from the elemental analysis of humic substances is the carbon and hydrogen content (Huffman and Stuber, 1985). The low levels of nitrogen generally found in humic substances lead to lower precision in its determination. The calculation of oxygen content based on the results of C,H and N measurement has a number of associated problems. First, this procedure magnifies the errors in the measurement of the other three elements. Also, the calculation assumes that no elements other than C,H,N and O are present in humic substances. This assumption can be considered a good approximation if the data available on sulfur and phosphorous are considered.

The sulfur content of humic substances has been reported by Schnitzer and Kahn (1972) to be generally near

zero. The average S content of aquatic humic substances has been reported to be between 0.96 and 0.40 percent (Thurman, 1985). Thurman (1985) points out that a S content of 0.6 percent for a humic substance with an average molecular weight of 1,500 would yield one sulfur atom per every four molecules of humic substances. Similarly, phosphorous has average values in aquatic humic substances of 0.1 to 0.4 percent (Thurman, 1985). Considering the low levels reported for these two elements, it is doubtful that the error of including them in the calculated oxygen content will create a significant error for each of the elemental analyses. It is also improbable that elements occurring at such low levels would have a major effect on the structure or behavior of the humic substances.

The data presented in Table 3.1 show a variation of elemental compositions for the humic substances investigated. The elemental compositions are within the ranges for individual elements reported in the literature. Trends in the elemental composition of humic substances from different sources have been reported (Stevenson, 1982: Steelink, 1985; Rice and MacCarthy, 1991). Humic acids generally have higher carbon and lower oxygen content than fulvic acids (Stevenson,1982; Rice and MacCarthy,1991). However, there is a great deal of overlap between the range of elemental compositions reported for these two groups.

Rice and MacCarthy (1991) have recently carried out an extensive review of the available elemental data reported for humic substances. The ranges reported for humic and fulvic acid percent carbon were 37.2-75.8 and 35.1-75.4, respectively. These ranges were obtained from 410 different humic acids sources and 214 fulvic acid sources. This compilation of available elemental composition data for humic substances is by far the most extensive review of its type ever undertaken. It should be noted that Rice and MacCarthy (1991) found sulfur data reported for only 160 of the 410 humic acids and 71 of the 214 fulvic acids. Although the ranges of carbon content reported for this large data base were wide, the observed standard deviations for the data sets were unexpectedly small. The mean values and 95 percent confidence intervals (mean ± 1.97 standard deviations) for elemental compositions of the humic substances reviewed by Rice and MacCarthy (1991) appear in Table 3.1. The values for the humic substances investigated in this dissertation fall within the 95 percent confidence interval for all samples except for the low percent carbon and high percent oxygen values for Newnans Lake 16 and 18 sediments. The hydrogen and nitrogen composition of the Newnans Lake Sediment samples are among the highest values obtained in this study, perhaps reflecting the high eutrophic nature of this lake. Similar elemental compositions have been reported for sedimentary humic

substances (Steelink, 1985). The higher nitrogen content of sedimentary humic substances may be a result of the relative decrease of lignin and an increase of amino acids as humic substance precursors in these systems (Steekink, 1985).

Ratios of elemental composition are often used to compare different humic substances (Stevenson, 1982; Ertel and Hedges, 1983; Steelink,1985; Rice and MacCarthy, 1991). Therefore, the atomic ratios have been included with the elemental composition data presented in Table 3.1. Again, the means and 95 percent confidence intervals for humic and fulvic acids reported by Rice and MacCarthy (1991) are included for comparison. The most common atomic ratios reported for humic substances are H/C and O/C. The H/C ratio has often been used to indicate the degree of unsaturation or aromaticity of humic substances(Rice and MacCarthy, 1991; Thurman, 1985). A small H/C ratio may indicate that the humic substance contains a large number of carbon to carbon double bonds. This reasoning has been questioned recently because it does not consider the contribution of carbon to oxygen double bonds to the H/C ratio (Rice and MacCarthy, 1991; Gauthier *et al.*, 1987; Perdue, 1984).

The ratios reported in Table 3.1 for the humic substances investigated in this dissertation fall within the 95 percent confidence intervals reported by Rice and MacCarthy (1991) except for the O/C ratios of Newnans Lake

16 and 18 sediment samples. As noted earlier, sedimentary humic substances have been reported to have higher H/C and O/C ratios than soil humic acids (Steelink, 1985; Ishiwatari, 1985). This has been postulated to be due to the difference in source material for lake sedimentary humic substances. The more aromatic lignin available in terrestrial environments is generally present in lakes through runoff and detritus (Stevenson, 1982). However, an eutrophic lake such as Newnans Lake (Göttgens, 1992) would be expected to contain significant aliphatic sources of humic precursors from the water column phytoplankton. The relative increase in the carbohydrate and amino acid content of the humic substance source materials would also account for the higher O/C ratio and higher nitrogen content of the Newnans Lake sedimentary humic substances. Lake sediment humic substances tend have aromatic ratios more similar to marine humic substances than to terrestrial humic substances (Ishiwatari, 1985; Rice and MacCarthy, 1991). The higher the amount of autochthonous material that contributes to the formation of lake sediment humic substances, the more aliphatic are the humic substances formed (Ishiwatari, 1985).

The elemental ratios obtained in this study for Aldrich humic acid compare well with those reported by Steelnik (1985) for an Aldrich humic acid. However, Steekink (1985) did not include the lot number for the Aldrich humic acid

used, therefore it was not possible to determine if it was the same lot that was used in this dissertation research. The H/C atomic ratio reported by Steelink (1985) was 0.80 compared with 0.84 measured in this research. The N/C atomic ratio of 0.01 reported by Steelink (1985) is the same value found for this ratio in the current study. However, the O/C atomic ratio of 0.66 measured in this study is different from the 0.46 value reported by Steelink (1985) and may indicates that the Aldrich humic acids are from different sources or atleast different lot numbers.

Based on comparison with the data available in the literature, the elemental compositions of the humic substances investigated for this dissertation (Table 3.1) are typical of humic substances from different sources.

Ultraviolet/Visible Spectroscopy

The relative simplicity, wide availability and small amount of sample required for this analysis have led to the wide application of ultraviolet/visible (UV/Vis) spectroscopy to investigations of humic substances (Schnitzer and Khan, 1972). The UV/Vis spectra of humic substances from both soil and aquatic environments have been found to be relatively featureless (Schnitzer, 1978; Stevenson, 1982) with absorptivity increasing as the wavelength of radiation deceases (Ertel and Hedges, 1983). This limits the application of UV/Vis spectra for the direct

determination of humic substances' structure (MacCarthy and Rice, 1985). However, the ratio of absorbances of a given humic substance at different wavelengths has been correlated with a variety of structural properties for different humic substances (Chen *et al.*, 1977).

Absorbance measurements were recorded at 254, 272, 465 and 665 nm for all humic substances investigated. The 254 and 272 nm spectral regions include $\pi \rightarrow \pi^*$ electronic transitions for phenolic arenes, benzoic acids, polyenes, and polynuclear aromatic hydrocarbons (Silverstein and Bassler, 1967). All these organic structures are known components of most humic substances (Novak *et al.*, 1992). The ratios of absorbances at 465 and 665 nm have been labeled the E_4/E_6 ratio by soil scientists and have been investigated extensively for humic substances (Chen *et al.*, 1977; Schnitzer, 1978).

UV/Vis spectra of an individual humic substances have been found to follow the Beer-Lambert Law for the relationship between the concentration of the humic substance and the absorption at a particular wavelength of radiation (Black and Christman, 1963; Stevenson, 1982). The relationship is given by:

$$A = abc \qquad (3.1)$$

where A is the absorbance at a particular wavelength, a is the absorptivity, b is the path length of light through the sample, and c is the concentration of the chromophore in the sample. The absorptivity of humic substances is generally reported in units of L/(g humic carbon·cm) (Stevenson, 1982; Gauthier *et al.*, 1987; Traina *et al.*, 1990; Novak *et al.*, 1992). The absorptivity of humic substances at a particular wavelength has been reported to vary with pH (Chen *et al.*, 1977; MacCarthy and Rice, 1985; Thurman, 1985). A maximum in the E_4/E_6 ratio has been seen for a variety of humic substances between pH 7 and 8 (Chen *et al.*, 1977). A review of the literature reveals that UV/Vis spectra of humic substances are often recorded from solutions at or near pH 7 in either 0.05 M NaCl (Traina *et al.*, 1990; Novak *et al.*, 1992) or 0.05 M $NaHCO_3$ (Chen *et al.*, 1977; Ertel and Hedges, 1983). Since the desired use of the UV/Vis absorbance data was to compare results among the different humic substances in this investigation and with work reported elsewhere, all measurements were made at pH 7.

Methods

The UV/Vis absorbance values were recorded using a Perkin Elmer Model 552 double beam grating spectrophotometer. Solutions were prepared for UV/Vis spectroscopy by dissolving approximately 10 mg of each humic substance in 10 mL of 0.01 M NaCl (Fisher Scientific Co.,

ACS Certified, Cat. no. S271-500). The pH of the solution was adjusted to 7 using 0.01 M HCl (Fisher Scientific Co., ACS Reagent, Cat. no. A144-212) or 0.01 M NaOH (Fisher Scientific Co., ACS Reagent, Cat. no. S320-500) (Traina *et al.*, 1990). Measurements were performed using 1.0 cm cells (220 nm cut-off, Fisher Scientific Co., Cat. no. 14-385-910C) with 0.01 M NaCl, pH 7 as a reference. Absorbance values were recorded for each humic substance at 254, 272, 465 and 665 nm against the 0.01 M NaCl reference. The absorptivities for each humic substance at the four wavelengths were calculated using equation 3.1. The E4/E6 ratio was calculated by dividing the absorptivity at 465 nm by the absorptivity at 665 nm (Schnitzer and Khan, 1972; Stevenson, 1982).

Results

The Beer-Lambert Law has been used for quantitative analysis for individual humic substances (Schnitzer and Kahn, 1972; Stevenson, 1982). The absorptivity at a particular wavelength for humic substances has been found to vary for different sources (Thurman, 1985; Gauthier *et al.*, 1987; Traina *et al.*, 1990; Novak *et al.*, 1992). This variation been attributed to differences in the structure and functional group composition of the humic substances (Stevenson, 1982; MacCarthy and Rice, 1985; Traina *et al.*, 1990).

The relatively featureless nature of UV/Vis spectra of humic substances has been attributed to the fact that they are complex mixtures of molecules which contain a number of chromophores per molecule (MacCarthy and Rice, 1985). A linear relationship (r^2 = 0.88) between the absorptivity at 272 nm and the percent aromatic carbon (determined by ^{13}C NMR) has been reported for 12 soil humic substances (Traina *et al.*, 1990). A more recent report comparing the same two parameters for eighteen terrestrial and aquatic humic substances found a weaker correlation (r^2 = 0.40) (Novak *et al.*, 1992). When the soil humic substances were considered alone, the correlation increased (r^2 = 0.72). There are a large number of functional groups in addition to aromatic moieties that are known to absorb light at or near 272 nm (Gauthier *et al.*, 1987). Since the absorptivity measured for a particular humic substance at 272 nm represents an average of all chromophores present, it is not surprising that the correlation with percent aromatic carbon reported by Novak *et al.*, (1992) is weak.

The decrease in the correlations discussed above with an expanded data set underscores an important consideration in humic substance research. When drawing general conclusions about humic substances from small data sets, one should be very cautious. The strength of large data sets, such as the one discussed earlier for elemental analysis

(Rice and MacCarthy, 1991), is that they allow correlations to be identified that are more statistically significant.

Traina *et al.* (1990) also reported elemental ratios for the humic substances investigated by UV spectroscopy and ^{13}C NMR. Combining their data with the data reported by Gauthier *et al.* (1987) these authors reported a weak correlation ($r = 0.42$) between the percent aromatic carbon and the atomic H/C ratio. Since both studies indicated a correlation between absorptivity of humic substances at 272 nm and percent aromatic carbon, Traina *et al.* (1990) concluded that perhaps a relationship exists between the absorptivity at 272 nm and the atomic H/C. Therefore, the data for the humic substances investigated in dissertation research (Tables 3.1 and 3.2) were combined with the data of Traina *et al.* (1990) and Gauthier *et al.* (1987) to form a data set which contained 35 different humic substance sources. These sources included humic substances from soils, marine and freshwater sediments as well as aquatic humic substances.

Data from three independent sources indicate that absorptivities increase as the humic substance H/C atomic ratios decrease (Figure 3.1). The correlation ($r = 0.62$, $p < 0.01$) was stronger than that reported by Novak *et al.* (1992) for the correlation ($r = 0.40$) of percent aromatic carbon with absorptivity at 272 nm for a diverse group of humic substances. This finding suggests that the

Table 3.2. Ultra-violet and visible spectral analysis results of humic substances.

HUMIC SOURCE	ABSORPTIVITY ($L \cdot gC^{-1} \cdot cm^{-1}$)				E4/E6
	254 nm	272 nm	465 nm	665 nm	
Aldrich Humic Acid	74.72	67.76	2.81	1.26	2.23
IHSS Humic Acid	76.42	71.58	3.66	0.99	3.69
Pine Mt. Soil	22.69	19.46	2.41	0.30	8.14
Orange Hts. Soil	63.29	55.59	2.05	0.48	4.28
Newnans Lake Sediment 11	36.03	31.97	1.41	0.40	3.53
Newnans Lake Sediment 16	33.50	30.16	1.13	0.31	3.65
Newnans Lake Sediment 18	35.57	31.79	1.76	0.43	4.11
Santa Fe River DOC	46.53	39.90	2.88	0.40	7.21
St. Marys River DOC	53.12	46.48	2.72	0.56	4.85
Orange Hts. DOC	47.49	40.80	2.42	0.47	5.15
Newnans Lake DOC	46.21	39.95	1.84	0.36	5.06
Suwannee River Fulvic	39.86	33.80	4.71	0.66	7.14
Suwannee River Humic	60.65	53.55	2.54	0.62	4.07

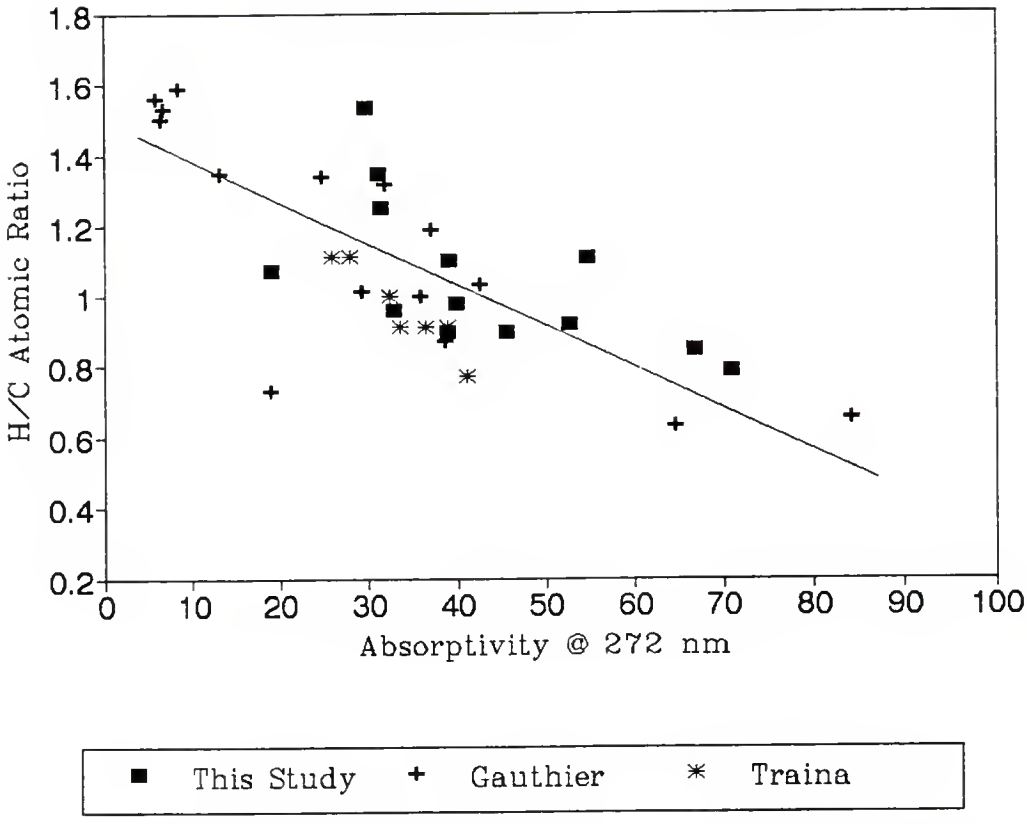

Figure 3.1. Absorptivity ($L \cdot gC^{-1} \cdot cm^{-1}$) at 272 nm was correlated with the H/C atomic ratio (r^2 = 0.64, $p < 0.01$) for the combined data of this study, Gauthier et al. (1987), and Traina et al. (1990).

absorptivity at 272 nm includes information about the concentration of other functional groups in addition to aromatic carbon.

Since both -HC=CH- and -COOH have H/C atomic ratios of one, the presence of carboxylic acid functional groups would adversely affect the use of the H/C atomic ratio as a measure of aromatic carbon content (Gauthier *et al.*, 1987). However, both these functional groups absorb at or near 272 nm. The data presented in Figure 3.1 support the conclusion that the atomic H/C ratio is not a good measure of the aromatic carbon content of humic substances. Further, the correlation between the absorptivity at 272nm and the H/C atomic ratio is likely the result of the combined affects of both aromatic and carboxylic acid functional groups.

The E_4/E_6 ratio has been used to characterize humic substances extracted from soil (Schnitzer and Khan, 1972; Chen *et al.*, 1977; Schnitzer, 1978; Stevenson, 1982) and isolated from water (Thurman, 1985). The E_4/E_6 ratio for a variety of humic substances has been shown to remain constant over a wide range of concentrations (Chen *et al.*, 1977). The ratio varies for humic substances extracted from different sources (Schnitzer and Khan, 1972). The ratio has been reported to be in the range of 2 to 5 for soil humic acids, 6 to 8.5 for soil fulvic acids (Schnitzer and Khan, 1972) and 5 to 22 for aquatic fulvic acids (Thurman, 1985).

The E_4/E_6 ratio has been correlated with a variety of compositional and structural characteristics of humic substances. It has been widely reported to be inversely correlated with the degree of condensation or aromatic carbon content of humic substances (Schnitzer and Khan, 1972; Schnitzer, 1978; Stevenson, 1982): lower ratios reflect higher amounts of aromatic carbon. The extensive study by Chen *et al.* (1977) of 15 different soil humic substances , however, found no evidence to support this hypothesis. For the humic substances investigated by Chen *et al.* (1977), the strongest correlation ($r^2 = 0.90$, $p < 0.01$) was found to be with the particle size and molecular weight of the humic substances. Weaker correlations were found between the E_4/E_6 ratio and the percent carbon ($r^2 = -0.53$, $p < 0.05$), percent oxygen ($r^2 = 0.67$, $p < 0.01$) and total carboxylic acid functional groups ($r^2 = 0.40$, $p < 0.05$).

The E_4/E_6 values of the soil and sediment humic substances measured in this study (Table 3.2) fall within the range for humic acids (Stevenson, 1982; Thurman, 1985) with the exception of the Pine Mt. Soil extract. This peat soil has a relatively high E_4/E_6 ratio. High E_4/E_6 ratios have been reported for other peat humic acids (Ertel and Hedges, 1983). The E_4/E_6 ratios for the aquatic humic substances fall in the range reported for fulvic acids (Schnitzer, 1978; Thurman, 1985) except for the St. Marys

River DOC sample. The value for this sample falls very near the lower limit generally attributed to aquatic fulvic acids (Thurman, 1985). The St. Marys River DOC sample was obtained at a time when the river was at flood stage. This may account for its E_4/E_6 ratio being between humic and fulvic acid values. The E4/E6 ratios measured for the humic substances in the current study indicate that the soil and sediment humic substances are predominately humic acids and the aquatic humic substances are predominately fulvic acids. This is consistent with reported compositions of humic substances from these sources. Extractable humic substances from soil are predominately humic acids (Stevenson, 1982) and aquatic humic substances are generally 85 to 90% fulvic acids in lakes and greater than 80% fulvic acids in streams and rivers (Thurman, 1985).

The relationship between the E_4/E_6 and humic substance structure and composition is unclear. Ertel and Hedges (1983) reported a linear correlation ($r^2 = 0.85$) between the E_4/E_6 ratio and the H/C atomic ratio for seventeen terrestrial and synthetic humic acids. The E_4/E_6 ratios for the humic substances investigated in this dissertation (Table 3.2) are also correlated with the H/C atomic ratio. This relationship is further illustrated by combining data of Ertel and Hedges (1983) and from Nissenbaum and Kaplan (1972) with the results from this dissertation reserach (Figure 3.2). The correlation between the E_4/E_6 ratio and

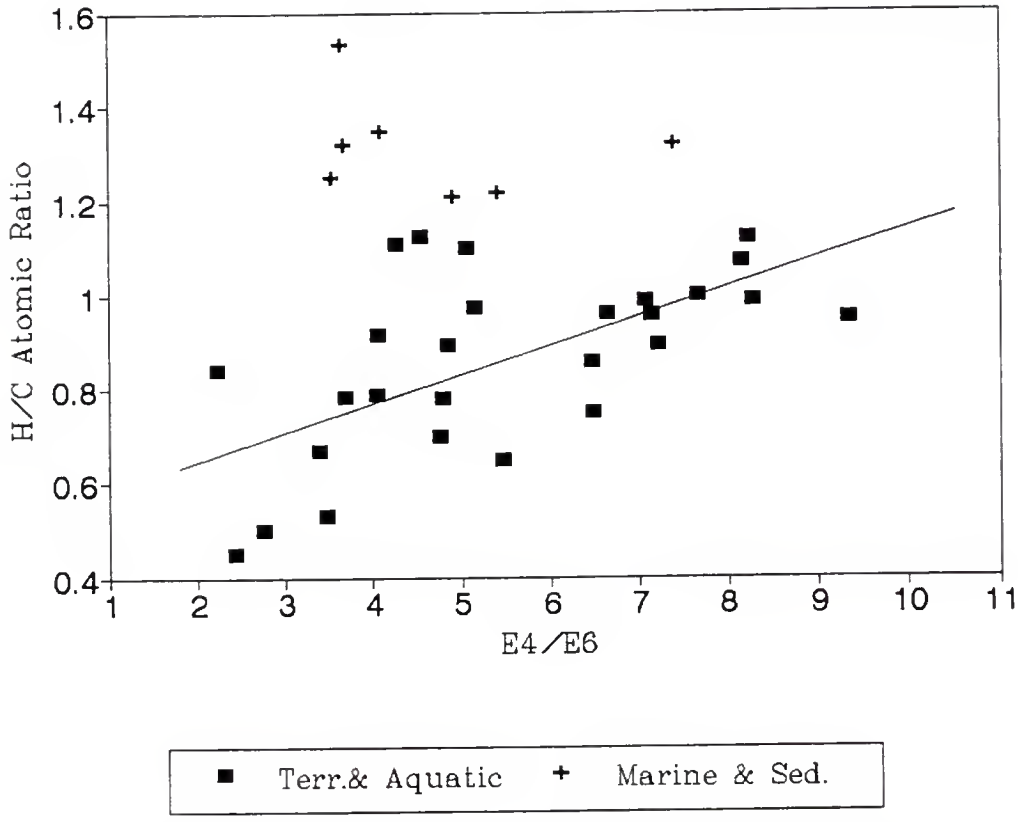

Figure 3.2. The E4/E6 ratio was correlated with the H/C atomic ratio (r^2 = 0.50, $p < 0.01$) for the combined data from this study, Nissenbaum and Kaplan (1972), and Ertel and Hedges (1983). Marine and sedimentary humic substances have been reported to have high H/C atomic ratios compared with their E_4/E_6 ratios (Ertel and Hedges, 1983).

the H/C atomic ratio (Figure 3.2; $r^2 = 0.49$, $p < 0.01$) was attributed by Ertel and Hedges (1983) to be the result of decreasing aromatic carbon content of humic substances as the H/C atomic ratio increased. However, data presented earlier for the relationships between the H/C atomic ratio and absorptivity at 270 nm (Figure 3.1) indicate that the H/C atomic ratio is a composite value resulting from a number of functional groups, including aromatic carbon and carboxylic acids. The weak relationship between H/C atomic ratio and the aromatic carbon content of humic substances reported by Traina *et al.* (1990) further illustrates the composite nature of the H/C atomic ratio. This leads to the conclusion that the E4/E6 ratio is likely the result of multiple functional groups.

The marine sediment humic acids and the Newnans Lake sediment humic substances appear above the other humic substances in Figure 3.2. Ertel and Hedges (1983) found that marine humic acids had low E_4/E_6 ratios compared to their H/C atomic ratios. This was attributed to reported low E_4/E_6 ratios for marine humic substances due to pigment absorption near 665 nm (Nissenbaum and Kaplan, 1970). Marine humic substances have higher H/C atomic ratios than their terrestrial counterparts (Rice and MacCarthy, 1991) Also, as noted earlier, lake sedimentary humic substances are similar to marine humic substances in their H/C atomic ratios (Ishiwatari, 1985). The higher H/C atomic ratios for

lake sedimentary humic substances and lower E_4/E_6 ratios for marine sedimentary humic substances may explain low E_4/E_6 ratios for the Newnans Lake Sediment samples compared to their H/C atomic ratios.

Although the UV/Vis spectra of humic substances do not yield direct information about their structure, the data presented here and in the literature indicate that there are relationships between the composition of humic substances and their UV/Vis spectra. Both the absorptivity at 272 nm and the E4/E6 ratios appear to be the result of multiple functional groups, including aromatic and carboxylic acid carbon. Further, the E4/E6 ratio for marine and sedimentary humic substances, when compared with their elemental composition, suggest that these two types of humic substances may be similar in their structure as well as their composition.

Total Acidity and Copper Binding Capacity of Humic Substances

The ability of humic substances in both the condensed and dissolved phases to complex metal ions has received a great deal of attention in recent years. Humic substances have been reported to complex both divalent and trivalent metal ions (Stevenson, 1982). The metal ions most strongly bound by humic substances are reported to be Hg^{+} and Cu^{++} (Mantoura *et al.*, 1978; Schnitzer, 1978; Stevenson, 1982; Thurman, 1985). Humic substances have been shown to control

the bio-availability of metals in soils and aqueous environments (Stevenson, 1982; McKnight *et al.*, 1983). Metal-humic substance interactions have also been reported to decrease the toxicity of heavy metals in solution (Tuschall and Brezonik, 1983).

The oxygen containing functional groups are believed to be the functional groups primarily responsible for metal ion binding by humic substances (Schnitzer and Khan, 1972; McBride, 1978; Stevenson, 1982) . Both carboxylic acids and phenolic hydroxyl groups have been reported as ligands involved in these complexing reactions (Vinkler *et al.*, 1976; Lakatos *et al.*, 1977; Piccolo and Stevenson, 1982; Stevenson, 1985). The acidic character of humic substances has also been attributed to these functional groups (Perdue, 1985; Thurman, 1985). Therefore, the total acidity and the copper binding capacity of the humic substances were investigated as measures of their oxygen containing functional groups.

Total Acidity

A large number of methods have been used for the analysis of acidic functional groups in humic substances (Schnitzer and Khan, 1972; Davis, 1982; Stevenson, 1982; Perdue, 1985). These include direct titrations, discontinuous titrations, indirect titrations, direct titrations coupled with distillation or ultrafiltration,

nonaqueous titration, irreversible reactions of acidic hydrogens with various reagents and other methods (Stevenson, 1982). The barium hydroxide ($Ba(OH)_2$) total acidity titration, which is an indirect titration method (Stevenson, 1982), was originally developed for brown coal and was adapted for humic substance research by Schnitzer and co-workers (Schnitzer and Gupta, 1965; Schnitzer and Khan, 1972). The $Ba(OH)_2$ titration method is by far the most often applied method for total acidity measurement of humic substances (Perdue, 1985) and was chosen as the method for this dissertation research.

The $Ba(OH)_2$ titration is based on the extreme insolubility of barium salts. In this method, the humic substance is allowed to equilibrate with a solution containing $Ba(OH)_2$. Humic substances containing acidic functional groups react with the Ba^{++} ions and precipitate. The samples are filtered to remove the precipitate and the resulting filtrate is titrated with HCl to a pH of 8.4. The total acidity of the humic substance is calculated by difference based on the initial $Ba(OH)_2$ concentration and the equilvalents of $Ba(OH)_2$ remaining in the filtrate.

Methods

In my research, 50-100 mg of each humic substance was allowed to equilibrate for 24 hours with 20 mL of 0.1 M $Ba(OH)_2$ (Fisher Scientific Co., Cat. no. B46-250) under a

nitrogen atmosphere. During this period, humic substances containing acidic functional groups reacted with the Ba^{++} ions and precipitated. A blank sample which consisted of 20 mL of $Ba(OH)_2$ was also allowed to equilibrate under the same conditions for the same period of time. The samples were then filtered (Whatman no. 41, ashless filter paper) to remove the precipitated Ba-humic acid. The filter was then thoroughly rinsed with CO_2 free DI water. The resulting filtrate and rinse water were combined and titrated with 0.050 M HCl to a pH of 8.4. The difference between the volume of titrant required by the sample and the blank titration was used to calculate the amount of $Ba(OH)_2$ remaining in solution.

The total acidity of the humic substance investigated was calculated using the following equation:

$$\text{meq } H^+/\text{g humic sub.} = (V_b - V_s) \text{ X M HCl/g humic} \quad (3.2)$$

where meq H^+ is the total acidity per gram of a specific humic substance, V_b and V_s are the volumes of titrant required to titrate the blank and sample, respectively. The total acidity values measured for the humic substances (Table 3.3) were normalized for the weight of humic substance and the weight of carbon for each source. Each humic substance was titrated in duplicate and the difference between replicate titrations was generally less than 5%

Table 3.3. Total acidity of humic substances.

Humic Source	$meqH^{+} \cdot gH^{-1}$	$meqH^{+} \cdot gC^{-1}$
Aldrich Humic Acid	7.37	14.50
IHSS Humic Acid	6.23	10.85
Pine Mt. Soil	4.59	7.80
Orange Hts. Soil	8.90	18.66
Newnans Lake Sediment 11	9.91	21.53
Newnans Lake Sediment 16	9.03	22.28
Newnans Lake Sediment 18	8.42	19.88
Santa Fe River DOC	8.40	17.00
St. Marys River DOC	9.24	18.06
Orange Hts. DOC	7.83	15.50
Newnans Lake DOC	7.61	15.15
Suwannee River Fulvic Acid	8.20	15.25
Suwannee River Humic Acid	6.89	12.72

(average, 3.7%; maximum, 9.9%, minimum, 0.1%). The mean variation for four replicate blank titrations was 1.0%.

Results

There has been some debate about the validity of the $Ba(OH)_2$ method for determining the total acidity of humic substances (Davis, 1982; Stevenson, 1982; Perdue, 1985). The presence of CO_2 will cause the measured total acidities to be high due to the precipitation of $BaCO_3$ during the equilibration period. However, this can be avoided by working under a nitrogen environment. In addition, there is some debate over whether or not the less acidic functional groups (i.e. phenols) react with the Ba^{++} (Stevenson and Gupta, 1965; Davis, 1982; Stevenson, 1982). The pK_a values for substituted phenols have been reported to be as high as 13, but most are near 10 (Perdue, 1985; Thurman, 1985). The $Ba(OH)_2$ solution has a pH greater than 13, which should assure the reaction of the phenolic ions. Recent reports of the direct potentiometric titration of aquatic humic substances have shown that less than 17% of their total acidity occurs at pK_a values greater than 10.3. Therefore, the values obtained from the $Ba(OH)_2$ method for total acidity of humic substances should yield reasonable estimates (Perdue, 1985).

The total acidity values measured in this research (Table 3.3) are within the ranges reported for humic

substances (Schnitzer and Khan, 1972; Stevenson, 1982; Perdue, 1985). The value of 8.2 meq H^+/gH obtained for the Suwannee River Fulvic Acid sample is very close to the reported value of 8.3 meq H^+/g measured by potentiometric titration (Bowles *et al.*, 1989). Fulvic acids and aquatic humic substances generally contain more acidic functional groups than soil derived humic substances (Schnitzer and Khan, 1972; Thurman, 1985), although the ranges reported for different types of humic substances overlap (Stevenson, 1982). The total acidity values of the aquatic humic substances measured were higher than the values for the soil derived humic substances, with the exception of the Orange Heights Soil sample. The sediment derived humic substances all had high total acidity values. The higher total acidity values measured for the aquatic humic substances in this study, compared with the soil humic substances, are consistent with the conclusion based on the E4/E6 ratios that the aquatic humic substances are predominately fulvic acids and the soil humic substances are predominately humic acids.

The total acidities of all humic substances investigated varied linearly (Figure 3.3; $r^2 = 0.83$, $p < 0.01$) with the O/C atomic ratio. The acidity of humic substances has been attributed to the oxygen containing functional groups (Schnitzer, 1972; Thurman, 1985), the majority of which are reported to be carboxylic acid and

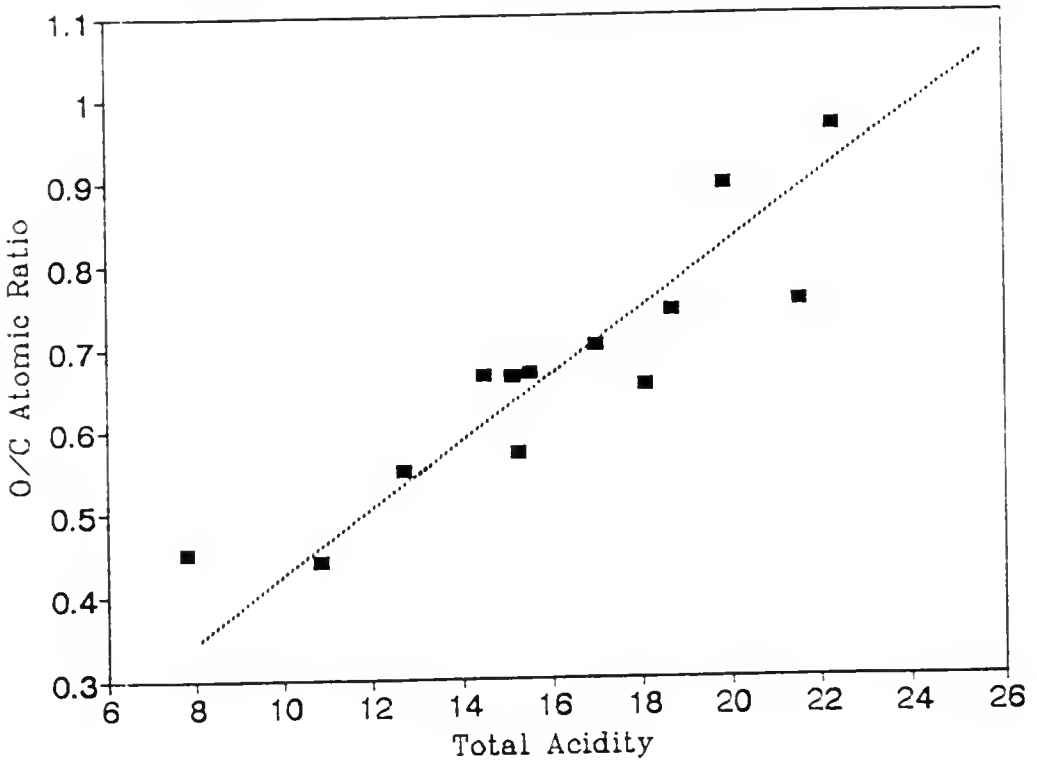

Figure 3.3. The O/C atomic ratio was linearly correlated (r^2 = 0.83, $p < 0.01$) with the total acidity (meq $H^+ \cdot g\ Carbon^{-1}$) measured for the humic substances in the current study.

phenolic hydroxyl groups (Stevenson, 1982; Perdue, 1985). Non-acidic oxygen containing functional groups reported to be present in humic substances include ketone and aldyhr carbonyls, alcohol hydroxyls, and ether groups (Steelink, 1985). The relationship between the total acidity and O/C atomic ratio suggests that acidic functional groups are more predoment than other oxygen containing functional groups in the humic substances included in this dissertation research.

Copper Binding Capacity

A number of methods have been used to measure the metal binding capacities of humic substances. These include ion selective electrode potentiometry, anodic stripping voltammetry, fluorescence quenching spectrophotometry, ultrafiltration, liquid chromatography and equilibrium dialysis (Thurman, 1985). Each of these methods has advantages and disadvantages which have been discussed extensively (Sarr and Weber, 1982). The method employed in this dissertation research was the equilibrium dialysis titration method of Truitt and Weber (1981a).

Equilibrium dialysis titration was used to determine the copper binding capacity of humic substances. The method involves equilibrating an aqueous solution of copper ions and a particular humic substance with DI water held in a dialysis bag (Guy and Chakrabarti, 1976; Truitt and Weber, 1981a). The dialysis membrane allows the freely dissolved,

hydrated metal ions to pass between the two solutions, while the metal ions complexed to humic substances are retained outside the membrane. After reaching equilibrium, the concentration of metal in each solution is measured by atomic absorption spectrophotometry. The metal ion concentration inside the dialysis bag represents the freely dissolved metal concentration and the metal ion concentration outside the membrane is the total metal ion concentration (freely dissolved plus metal ion/humic complexes). The titration involves incremental addition of metal ions, equilibration and AAS measurement of the metal ion concentration in the two solutions. The addition of copper is continued until the complexing capacity of the humic substance is past saturation (Truitt and Weber, 1981a). A linear regression calculation of the free metal ion concentration versus the total metal ion concentration yields an intercept that represents the total complexing capacity of the humic substance.

Methods

The dialysis titration experiments were performed according to the method of Truitt and Weber (1981a). The Spectra/Por 6 dialysis tubing (molecular weight cut off of 1,000, Fisher Scientific Co., Cat. no. 08-670-12B), required cleaning before use to remove preservatives. The tubing was rinsed with DI water and then soaked in 0.1% Na_2S (Fisher

Scientific Co., Cat. no. S426-212) at 60 °C for 15 minutes. The tubing was then rinsed in warm DI water, soaked in 3% H_2SO_4 (Fisher Scientific Co., ACS Reagent, Cat. no. A300-212) at 60° C for 5 minutes, rinsed with DI water and stored in DI water until needed.

The dialysis titration experiments were performed in 1 L Nalgene bottles which had been rinsed with 50% nitric acid (Fisher Scientific Co., Cat. no. A202-212) to remove any metal ions. Solutions of humic substances (approximately 20 mg/L) were prepared in 0.001 M NaCl at pH 7. The pH of the solution was adjusted as needed using 0.1 M HCl or NaOH. Initially, 0.50 mL of 1000 mg/L Cu^{++} standard solution (Fisher Scienctific Co., Cat. no. SC194-100) was added to each humic substance solution to yield a solution concentration of 0.5 ppm Cu^{++}. The humic substance/copper ion solution was placed in the 1 L bottle and a dialysis bag containing 40 mL of 0.001 M NaCl at pH 7 was added and the bottle sealed. The bottle was placed on a shaker table overnight and then allowed to equilibrate for an additional day. Solutions were equilibrated for 48 hours between metal additions. This was probably more than sufficient time to achieve equilibration between the two solutions since Guy and Chakrabarti (1976) reported no change between the two solutions for a number of humic substances after about 20 hours. The equilibrated solutions were subsampled by removing 1 to 2 mL of the solutions from both inside and

outside the membrane. The copper concentration of each subsample solution was then determined by AAS. After samples were obtained, another aliquot of Cu^{++} standard solution was added to each humic substance solution and the equilibration procedure repeated. As mentioned earlier, the titration of Cu++ was complete when the humic substances were all past saturation (slope of titration curve is one) (Truitt and Weber, 1981a).

Each batch of dialysis titration experiments included a blank dialysis sample which consisted of a 0.001 M NaCl/Cu^{++} solution outside the dialysis bag and 0.001 M NaCl inside the bag. The blank samples were run for the duration of the humic titration experiments to confirm that freely dissolved Cu^{++} ions passed through the dialysis membrane and did not significantly adsorb to the membrane. In all cases, the Cu^{++} concentrations inside the dialysis tubing were found to be the same as solution concentrations outside the tubing. Also, no adsorption of metal to the dialysis membrane was observed since the concentrations of Cu^{++} measured in these blank samples was the same as predicted based on the amount of Cu^{++} added to the solutions.

Another potential problem with the dialysis titration technique is the leakage of humic substances across the dialysis membrane. Any leakage of humic substances across the membrane would lead to an underestimation of the Cu^{++} binding capacity. Truitt and Weber (1981a) noted that at pH

5 approximately 30% of humic substances investigated were able to pass through the dialysis membrane, while at pH 7 less than 5% passed through the membrane. These values were based on the percentage of the retentate color (UV absorbance at 260 nm) that diffused across the membrane. The lack of color of the retentate solutions after equilibrium in this research indicates that there was no leakage of humic substances across the dialysis membrane. This was in agreement with the reported low level of humic substances breakthrough at pH 7 (Truitt and Weber, 1981a).

The AAS analysis was performed using a Perkin Elmer Model 5000 AAS with an air/acetylene flame. The hollow cathode lamp (Perkin Elmer, Inc., Cat. no. 303-6024) was energized at 15 mA and the 325 nm copper emission was monitored in the absorbance mode using a 0.7 nm slit width. Each time samples were analyzed, a standard curve for Cu was constructed using the following concentrations of standards (in ppm Cu): 0.5, 2, 4, 6, 10. Typical calibration curves were linear ($r^2 \geq 0.99$) and indicated very little or no bias (i.e. intercepts at or near 0 ppm Cu and 0 absorbance units).

The Cu^{++} binding capacity was obtained by plotting the freely dissolved Cu^{++} concentration vs. the total Cu^{++} concentration (Guy and Chakrabarti, 1976; Truitt and Weber, 1981a). A typical dialysis titration plot is presented in Figure 3.4 for Newnans Lake DOC. It should be noted that

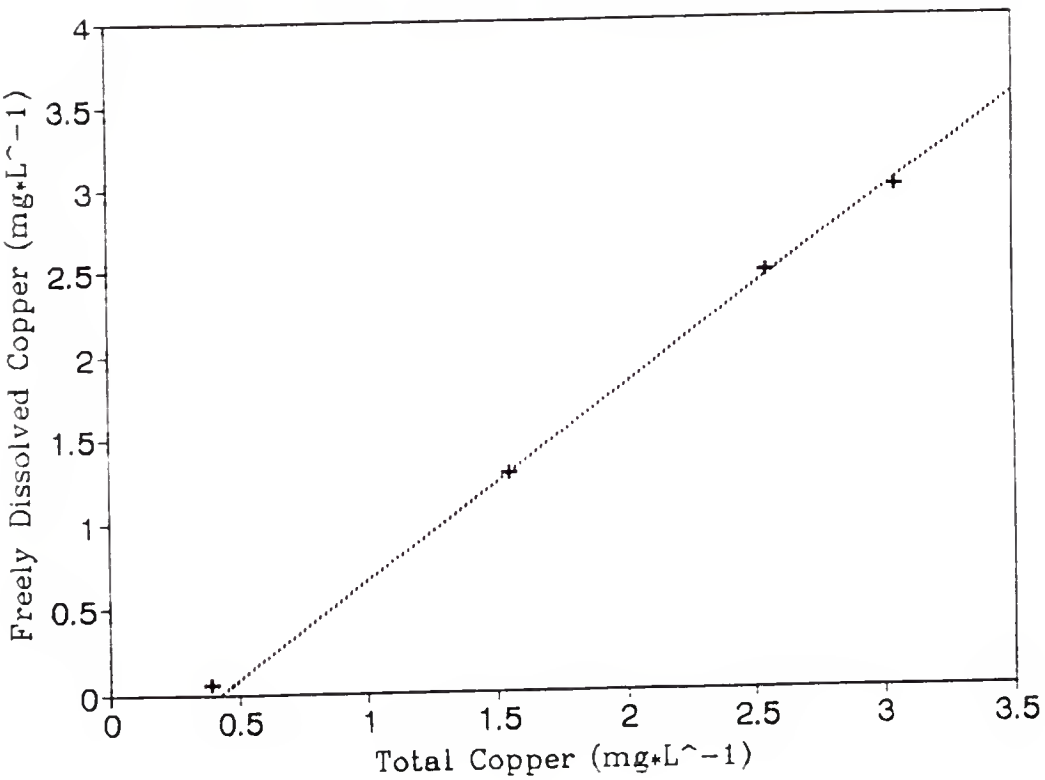

Figure 3.4. Copper dialysis titration for Newnans Lake DOC sample (20 mg humic·L^{-1}) at pH 7 and 0.001 M NaCl.

the first data point of the titration curve was below the Cu^{++} saturation point and that the linear portion of the curve above the Cu^{++} saturation point was extrapolated to the abscissa to obtain the Cu^{++} binding capacity (Guy and Chakrabarti, 1976).

Results

The Cu^{++} binding capacities of the humic substances measured in this study (Table 3.4) are in the range for Cu^{++} binding data previously reported for humic substances. McKnight *et al.*(1983) reported the Cu^{++} binding capacities for 18 humic substances isolated from surface waters using XAD-8 resin. They found copper binding capacities ranging from 0.54 to 2.55 μM Cu^{++}/mg C. Copper binding capacities found during this study cover a similar range and fall within the range of Cu^{++} binding capacities reported by McKnight, et al (1983).

The Cu^{++} binding capacity of the International Humic Substances Society's Suwannee River Fulvic Acid Reference (SRFAR) material has been reported to be 1.2 to 1.4 μM Cu^{++}/mgC in 0.001 M KNO_3 (McKnight *et al.*, 1983; McKnight and Wershaw, 1989). Ventry *et al.* (1991) reported the Cu^{++} binding capacity for this humic material to be between 4.6 and 6.3 μM Cu^{++}/mgC when measured in 0.1 M $NaClO_4$. The value measured for SRFAR measured in my research (0.70 μM Cu^{++}/mgC) is lower than either of these values. However,

Table 3.4. Copper binding capacity of humic substances.

HUMIC SOURCE	$\mu M\ Cu^{++} \cdot mg\ C^{-1}$	r^2
Aldrich Humic Acid	2.25	0.971
IHSS Humic Acid	0.7	0.997
Pine Mt. Soil	2.11	0.999
Orange Hts. Soil	1.24	0.999
Newnans Lake Sediment 11	0.91	0.997
Newnans Lake Sediment 16	1.04	0.999
Newnans Lake Sediment 18	1.2	0.997
Santa Fe River DOC	1.74	0.999
St. Marys River DOC	2.06	0.998
Orange Hts. DOC	1.64	0.999
Newnans Lake DOC	0.8	0.998
Suwannee River Fulvic Acid	0.7	0.999
Suwannee River Humic Acid	0.71	0.999

the value reported in Table 3.4 is closer to that reported by McKnight *et al.* (1983) who indicated that the value was probably good to within a factor of two. The values reported by Ventry *et al.* (1991) are high and are significantly beyond the ranges reported by either McKnight *et al.* (1983) or this study. Also, the conditional stability constants for Cu^{++} complexation by SRFAR reported by Ventry *et al.* (1991) were an order of magnitude lower than those previously reported for this humic substance (McKnight *et al.*, 1983; McKnight and Wershaw, 1989).

The reason for this discrepancy between reported copper binding capacities may be related to the ionic strength of the solutions used to make the measurements. The strength of the interactions between metal ions and humic substances (as measured by the conditional stability constant) decreases as the ionic strength is increased (Schnitzer and Khan, 1972). The measured Cu^{++} binding capacity of a humic substance has been reported to be dependent on the ionic strength of the solution (McKnight, 1983). The Cu^{++} binding capacity of SRFAR was observed to decrease from 1.4 μM Cu^{++}/mgC at 0.00001 M $Ca(NO_3)_2$ to 0.73 μM Cu^{++}/mgC at 0.01 M $Ca(NO_3)_2$ (McKnight and Wershaw, 1989). Also, the Cu^{++} binding capacities of six natural waters containing DOC showed strong negative correlations with alkalinity (r^2 = -0.91) and conductance (r^2 = -0.89) (Truitt and Weber, 1981b). Thus, the Cu^{++} binding capacity of SRFAR would be

expected to be lower in 0.1 M $NaClO_4$ than in either 0.001 M NaCl or 0.001 M KNO3. The ionic strength dependence of Cu^{++} binding capacity may also explain the difference between the SRFAR values measured by McKnight *et al.* (1983; 1989) and the value I obtained during this research. The pH of the solutions used in the dialysis titrations were adjusted with 0.1 M HCl and 0.1 M NaOH. The ionic strength of the solution could have been greater than 0.001 M and been closer to 0.01 M. The Cu^{++} binding capacity for SRFAR in 0.01 M $Ca(NO_3)_2$ has been reported to be 0.73 µM Cu++/mgC (McKnight *et al.*, 1989) which is close to the value of 0.70 µM Cu^{++}/mgC measured in the this study.

Copper is one of the most strongly bound metals when chelated in organic complexes with oxygen containing ligands (Schnitzer, 1978; Stevenson, 1982; Thurman, 1985). Humic substances have been reported to bind Cu^{++} more strongly than any other divalent cation with the exception of Pb^{++} (Guy and Chakrabarti, 1976). Mantoura *et al.* (1978) found that 99.9% of the copper present in lake and river waters was complexed to dissolved humic substances. Copper has been reported to displace more weakly bound cations when it complexes with humic substances (Truitt and Weber, 1981b; Stevenson, 1982; Albert *et al.*, 1989). The stronger retention of Cu^{++} compared with other metals (Mn^{++}, Mg^{++}, and Cr^{++}) has been investigated using electron spin resonance spectrometry (Lakatos *et al.*, 1977; McBride, 1978). The

results indicate that oxygen containing functional groups are able to form inner sphere complexes with Cu^{++}, but not with the other metal ions. This means that oxygens in humic substances displace the water of hydration when coordination complexes are formed with Cu^{++}.

The metal complexing behavior of humic substances has generally been attributed to oxygen containing functional groups. Carboxylic acids and phenolic hydroxyl groups are the most frequently mentioned functional groups responsible for these interactions (Schnitzer and Khan, 1972; Stevenson 1982; Thurman, 1985; Gamble *et al.*, 1970; McKnight *et al.*, 1983; McBride, 1978; Lakatos *et al.*, 1977; Vinkler *et al.*, 1976; Piccolo and Stevenson, 1982). Nitrogen containing functional groups are also capable of complexing metal ions and no doubt are responsible for a portion of the measured metal binding capacities of humic substances (Stevenson, 1982; Thurman, 1985). However, since the number of nitrogen containing functional groups in humic substances is small compared to the number of oxygen containing functional groups, the latter would be expected to play the dominant role in interactions with metal ions. The exception would be marine humic substances which often contain significant amounts of nitrogen (Harvey and Boran, 1985; Rice and McCarthy, 1991).

The mechanism of binding between humic substances and metal ions is not clear. Two main types of chelation

reactions between metals and humic substances have been proposed. These include the "salicylate-like" site which contains a carboxylic acid adjacent to a phenolic hydroxyl group and the "phthalate-like" site which contain ortho-carboxylic acid functions (Piccolo and Stevenson, 1982; Gamble, *et al*. 1970). Inter-molecular bidentate complexes between different humic molecules and a single metal ion also are likely to occur (Schnitzer, 1978). Vinkler *et al*. (1976) reported that metal ions interact predominately with carboxylate functional groups based on the IR spectra of humic substances and their metal complexes. However, another investigation indicated that humic substance metal binding reactions involved both carboxylate and hydroxyl functional groups (Gamble *et al*., 1970). This conclusion was based on the fact that the greatest reduction in Cu^{++} binding capacity for a series of humic substances was observed when both carboxylate and phenolic hydroxyl groups were chemically blocked. Blocking either one of these functional groups alone reduced the Cu^{++} binding capacities of the humic substances investigated, but to a lesser extent than when both groups were blocked. A similar study using functional group blocking concluded that the major reaction of metals with humic substances involves a phenolic hydroxyl and a carboxyl group (Schnitzer and Kahn, 1972). Another minor reaction involves two carboxylic acid functional

groups. Aliphatic hydroxyl groups were not found to be important in metal binding interactions.

The exact nature of metal/humic substances interactions is difficult to discern due to the complex nature of humic substances themselves. There is little disagreement that oxygen containing functional groups are important in these interactions (Stevenson, 1982; Thurman, 1985). This conclusion is supported by the data obtained in this research. The Cu^{++} binding data from Table 3.4 is plotted in Figure 3.5 against the H/O atomic ratio. There is a strong negative correlation ($r^2 = 0.88$, $p < 0.01$) between the Cu^{++} binding capacity and the H/O atomic ratio for the humic substances investigated in this study, with the exception of the Pine Mt. Soil humic substance. As noted in the earlier discussion of elemental composition in this Chapter, the Pine Mt. Soil sample appears to be an outlier with respect to its H/O atomic ratio. It is interesting to note that although the Cu^{++} binding capacities correlated well with the H/O ratio for the humic substances investigated, they did not correlate with the total acidity ($r = 0.16$, $p > 0.5$). Stevenson and Goh (1974) have reported free, uncomplexed carboxylic acid functional groups in the infrared spectra of humic substances which have been saturated with Cu^{++}. The data presented for the humic substances investigated in this dissertation research indicate that there is no simple relationship between the

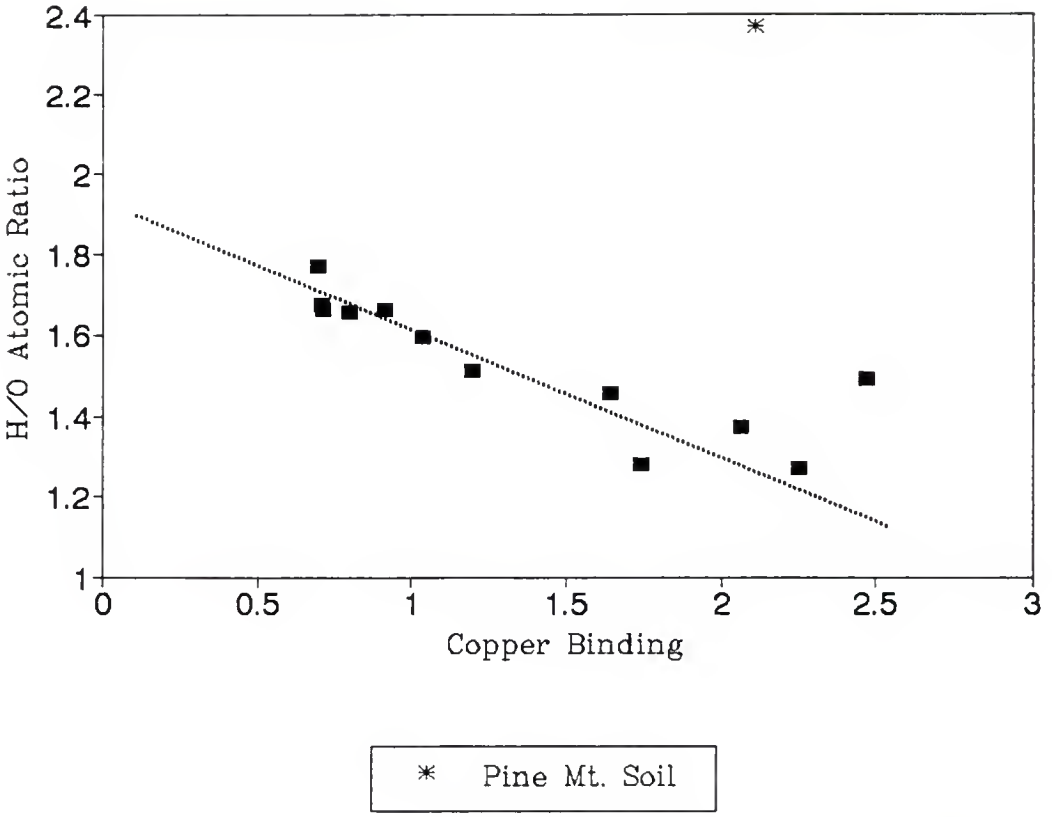

Figure 3.5. Copper binding capacity (μMole $Cu^{++} \cdot mg\ Carbon^{-1}$) was linearly correlated ($r^2 = 0.88$, $p < 0.01$) with the H/O atomic ratio for the humic substances in the current study. Pine Mt. Soil was excluded from the correlation calculation.

acidic functional group content and the Cu^{++} binding capacity. The lack of correlation between these two seemingly related parameters may be due to the fact that some acidic functional groups are inaccessible to Cu^{++}.

Infrared Spectroscopy of Humic Substances

Infrared (IR) spectroscopy has been widely used for the investigation of humic substances (MacCarthy and Rice, 1985; Lobartini and Tan, 1988) and has provided a great deal of information on the nature, reactivity and structural arrangement of oxygen containing functional groups in humic substances (Stevenson, 1982). Infrared spectra of humic substances are the result of the absorption of IR radiation by a complex mixture of molecules which are themselves multi-functional. The resulting IR spectra contain a variety of bands which are indicative of the different functional groups present in these complex mixtures (Stevenson, 1982). IR spectra have been used to investigate the interactions of humic substances with metals (Vinkler *et al.*, 1976; Piccolo and Stevenson, 1982; MacCarthy and Rice, 1985) and pesticides (Kahn, 1974; Kahn, 1978).

IR spectroscopy, like UV/Vis spectroscopy, is a quantitative technique that follows the Beer-Lambert Law (Schnitzer and Khan, 1972; Stevenson, 1982). The sample size required to obtain an IR spectrum is 1 to 10 mg (MacCarthy and Rice, 1985). This small sample size is an

advantage when IR spectroscopy is compared with ^{13}C NMR spectroscopy, which also provides information on functional groups, but requires sample sizes ≥ 100 mg (Wershaw, 1985). Fourier transform IR spectroscopy (FTIR) has a number of advantages compared to more traditional dispersive IR techniques. These advantages include better resolution, higher signal to noise ratios (due to spectra averaging) and higher energy throughput (MacCarthy and Rice, 1985). The increased resolution is not a great advantage in the current study due to the broad bands produced by humic substances (Schnitzer and Khan, 1972). However, the increased energy throughput and higher signal to noise ratio lead to increased sensitivity. Although FTIR instruments have been available for sometime, relatively little work with these instruments has been reported for humic substances.

The ease of obtaining an IR spectrum, coupled with the large amount of published information on the IR spectra of humic substances, make this an attractive technique for the investigation of the functional group composition of humic substances. The fact that IR analysis is potentially quantitative is also attractive, although, the quantitative application of IR spectroscopy to humic substances is rarely reported (Schnitzer and Khan, 1972; Stevenson, 1982).

The desired result of the application of IR spectroscopy in my study was to obtain quantitative measurements of specific functional group compositions among

the different humic substances investigated. Therefore, an internal standard FTIR method was developed for the investigation of humic substances. The technique involves use of an internal standard to compensate for the variation in sample presentation and instrumental variation between different samples. The internal standard approach allowed the direct comparison of the intensities of specific IR peaks among different spectra and therefore between different humic substances. The IR data obtained using this method allowed the absorption bands assigned to specific functional groups to be quantitatively compared.

Methods

Sample presentation is an important consideration in IR spectroscopy. The most common method of sample presentation for obtaining IR spectra of humic substances is the KBr pellet (Stevenson, 1982, MacCarthy and Rice, 1985). In this technique, pellets are made by mixing 1 to 2 mg of dried humic substance and 100 to 200 mg of Kbr (Stevenson, 1982; Schnitzer, 1978). The pellet is pressed into a disk. The disk is then illuminated with IR radiation and the spectrum recorded. These types of samples are used for qualitative comparisons among the functional groups of different humic substances. A disadvantage of this technique is that, under certain conditions, KBr has been reported to catalyze the formation of cyclic anhydrides from carboxylic acids, thus

altering the humic substances under investigation (Stevenson and Goh, 1974; Wagner and Stevenson, 1965).

Other techniques for sample presentation in IR spectroscopy include the preparation of mulls of samples with a hydrocarbon oil (Bellamy, 1975; MacCarthy and Rice, 1985) and cast films placed on IR transparent plates (MacCarthy and Rice, 1985). The mull technique has the disadvantage that the hydrocarbon oil used to prepare the mull absorbs strongly in the aliphatic carbon region of the spectrum, a spectral region of interest in this research. The cast film technique involves applying a solution of the humic sample to an IR transparent plate followed by evaporation of the solvent to dryness (MacCarthy and Rice, 1985). The IR spectrum is recorded from the dried deposit. Cast film deposits are less susceptible to adsorption of moisture than the more hydroscopic KBr pellets. The disadvantage of this method is that a satisfactory film is not formed for all materials. The cast film sample technique was chosen for this research because of the inherent homogeneity of liquid solutions. Homogeneity of the sample/internal standard solution is a basic requirement for the success of any internal standard analysis.

The optimum compound for an internal standard for IR analysis of humic substances would be one that has a single, strong absorption band in a spectral region where humic substances do not absorb. The compound must also be soluble

in the solvent used to make the film deposits and be unreactive with the humic substances. The spectra of humic substances are void of absorption bands between 2400 cm^{-1} and 1800 cm^{-1} (Stevenson, 1982). Potassium thiocyanate (KSCN) was chosen as an internal standard because it contained a strong absorption band at 2050 cm^{-1} (Sadtler Infrared Index, 1973) and was freely soluble in water and 50% dimethylsulfoxide (DMSO), the two solvent systems used to make film deposits.

Two solvent systems were used because cast films of all humic substances could not be obtained from a single solvent. It was found that the humic substances investigated all formed satisfactory films from aqueous solution at pH 11. However, at this elevated pH, all ionizable protons are dissociated, causing the carboxylic acid absorption band at approximately 1700 cm^{-1} to shift to lower frequencies indicative of the carboxylate anion (near 1600 cm^{-1} and 1400 cm^{-1}) (Piccolo and Stevenson, 1982). The 1600 cm^{-1} region of the spectrum is also where aromatic carbon absorption occurs (Eltantawy and Baverez, 1978) Therefore, IR spectra were recorded for all humic substances investigated on films deposited from aqueous solution at pH 11 and from 50% aqueous DMSO at pH 4. The latter solvent system was employed to obtain spectra of humic substances with carboxylic acids in the un-ionized form. Aqueous DMSO has been reported to extract humic substances from soils

which are identical in composition to the humic substances extracted with aqueous NaOH (Hayes, 1985).

Solutions for FTIR analysis were prepared by dissolving humic substances (approximately 10mg/mL) into a solution containing a fixed amount of KSCN (1.97 mg/mL) (Fisher Scientific Co., Cat. no. P317-100) in either DI water or 50% DI water/ 50 % DMSO (Fisher Scientific Co., Spectranalyzed Cat. no. D136-1). The pH of the solutions were adjusted with 0.001 M NaOH or HCl. Cast films were prepared by depositing 50 µL of the humic substance/internal standard solution onto a 3 mm thick ZnSe plate (Harrick Scientific Corp., Cat. no. WMD-U37) and placing the plate in a drying oven at approximately 70 °C for 15 to 20 minutes. The dried deposit was then placed in a sample holder with an adjustable aperture set to a diameter of 5.5 mm. The deposit was then placed into the sample chamber of the FTIR spectrometer and the chamber evacuated to a pressure of approximately 1-3 millitorr before the IR spectrum was recorded. The spectra were recorded using a Bomem DA3.10 FTIR spectrometer scanning over the frequency range from 4000 cm^{-1} to 400 cm^{-1} at a resolution of 2 cm^{-1}. The spectrometer was equipped with a Globar source, a Ge/KBr beam spliter and a liquid nitrogen cooled Hg/Cd/Te detector. To insure a high signal to noise ratio, the spectrum for each sample was calculated from the average of 100 repetitive scans.

Spectra were obtained from at least duplicate deposits for each humic substance at pH 4 and 11. The peak heights for each peak of interest were measured and normalized to the peak height of the internal standard peak at 2050 cm^{-1}. The data are reported as peak height per gram of humic substance normalized to the peak height at 2050 cm^{-1} per gram of KSCN (units for peak height ratio: g KSCN/g humic substance).

A number of conditions are required for the internal standard method to be successful. First, the internal standard and the humic substances must be uniformly mixed in the solution. Second, the internal standard/humic substance solution must form a uniform film when it is dried on the ZnSe plate. Finally, the internal standard must behave in a similar manner as the humic substances. In order to investigate whether the KSCN was suitable for use as an internal standard, experiments were performed which used potassium acid phthalate (KHP) as a model for humic substances. Solutions with varying KHP concentration (Table 3.5) and constant KSCN concentration were prepared and analyzed using the cast film method described above.

Results

The IR spectra obtained for the KHP were those expected from the literature (Sadtler Infrared Index, 1976). The peak heights and areas for the two major KHP peaks (1560 and

Table 3.5. Potassium hydrogen phthalate internal standard calibration.

	PEAK HEIGHT [1]					PEAK AREA [2]		
KHP	KHP		KSCN	KHP/KSCN		KHP	KSCN	KHP/KSCN
CONC. ($mg \cdot mL^{-1}$)	1560 (cm^{-1})	1410 (cm^{-1})	2050 (cm^{-1})	1560	1410	1410 (cm^{-1})	2050 (cm^{-1})	1410
3.86	155.8	156.4	117.9	1.166	1.228	31.20	3.90	8.000
3.86	211.6	237.2	207.3	1.021	1.144	29.50	3.76	7.846
3.86	212.4	236.3	208.5	1.019	1.133	22.10	2.94	7.517
1.93	239.0	206.0	393.0	0.608	0.524	27.05	8.84	3.060
1.93	177.0	189.0	334.0	0.530	0.566	23.92	8.09	2.957
1.93	205.0	191.0	413.0	0.496	0.462	15.27	5.24	2.914
0.97	88.1	56.1	260.0	0.339	0.216	6.90	5.59	1.234
0.97	76.5	75.3	298.0	0.257	0.253	8.98	7.30	1.230
0.97	48.6	71.6	201.0	0.242	0.356	7.49	3.55	2.110
0.48	52.9	54.0	277.0	0.191	0.195	7.11	7.11	1.000
0.48	44.3	56.5	299.0	0.148	0.189	6.71	6.32	1.062
0.48	45.3	57.5	280.6	0.161	0.205	5.58	5.44	1.026

[1] Absorbance ($\times 10^3$)
[2] Arbitrary units
[3] KSCN concentration was constant at 1.97 $mg \cdot mL^{-1}$

1410 cm^{-1}, COO^-) were measured and normalized to the peak height and area for the KSCN peak at 2050 cm^{-1} (Table 3.5). A strong linear correlation was found between the normalized peak area ratios and peak height ratios for the KHP peak at 1410 cm^{-1} (Figure 3.6, $r^2 = 0.98$, $p < 0.001$). A similar relationship was obtained for the KHP peak at 1560 cm^{-1} ($r^2 = 0.96$, $p < 0.001$). These results demonstrate that either the peak area or height ratio can be measured to yield the same result. The relative complexity of the IR spectra of humic substances, compared to that of KHP, led to the decision to measure peak height rather than peak area in their IR spectra. The normalized peak heights for the KHP peak at 1560 cm^{-1} were found to vary linearly with the KHP concentration (Figure 3.7, $r^2 = 0.97$, $p < 0.001$). It should be noted that these data (Table 3.5) demonstrate that the peak height for a given concentration of KHP or KSCN can vary by greater than 200%, but the variation in the KHP/KSCN ratio is less than 30% in all cases and generally below 15%. These data support the conclusion that KSCN is a suitable internal standard for the quantitative application of the IR cast film method used in the current research.

The final questions remaining for the application of the KSCN internal standard cast film method for the semi-quantitative IR investigation of humic substances are: whether or not the KSCN act in the same manner when mixed with humic substances as it did with KHP and whether

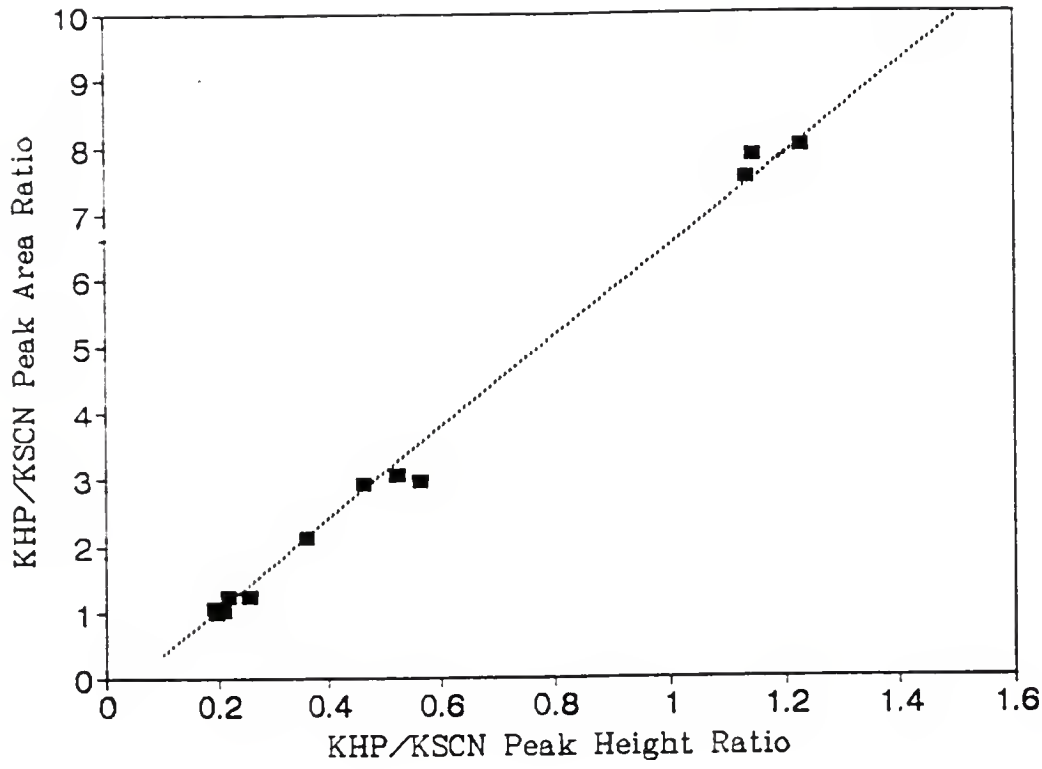

Figure 3.6. The peak height ratios and peak area ratios for the KHP peak at 1410 cm^{-1} and the KSCN peak at 2050 cm^{-1} were linearly related (r^2 = 0.98, $p < 0.01$). The KHP concentration varied from 0.48 to 3.86 $mg \cdot mL^{-1}$ while the KSCN concentration remained constant at 1.97 $mg \cdot mL^{-1}$.

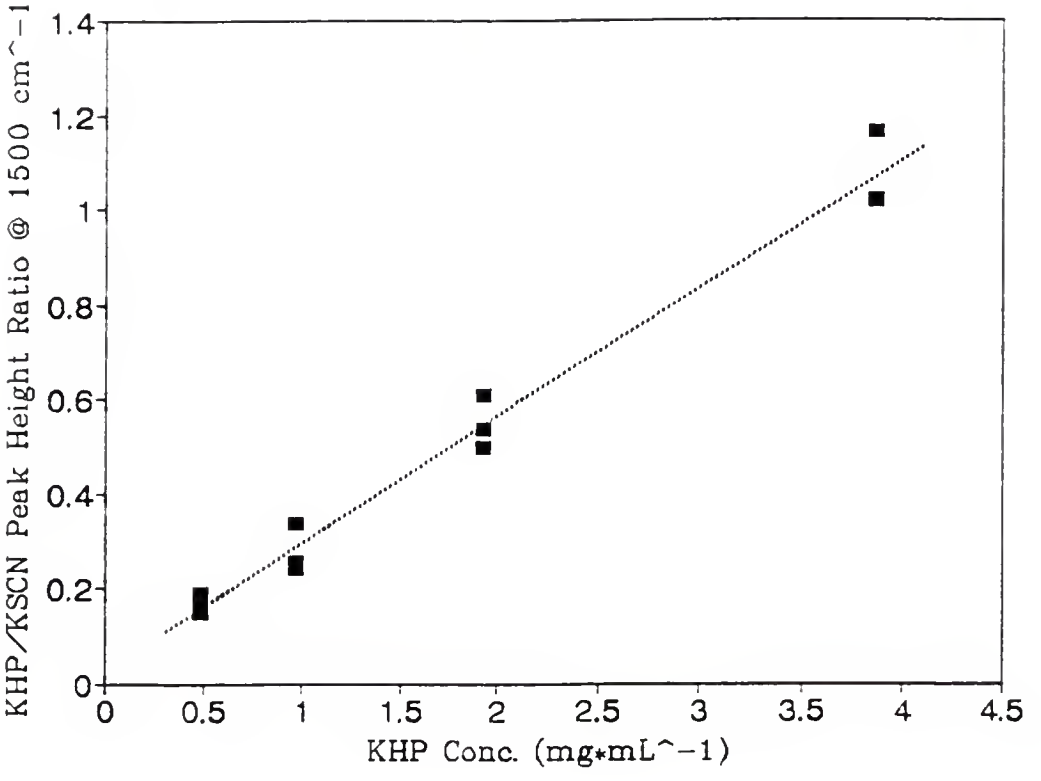

Figure 3.7. The KHP concentration ($mg \cdot mL^{-1}$) was correlated ($r^2 = 0.97$, $p < 0.01$) with the peak height ratio of the KHP peak at 1560 cm^{-1} to the KSCN peak at 2050 cm^{-1}. The KSCN concentration was constant at 1.97 $mg \cdot mL^{-1}$.

reproducible deposits of the KSCN/humic substance solutions can be made. Although the KSCN peak at 2050 cm^{-1} was present in all spectra obtained from deposits of humic substances, the possibility exists for a portion of the KSCN to be masked by interactions with the humic substances. One example of such an interaction would be H-bonding between the KSCN and polar humic functional groups. These interactions could cause either band broadening and/or a frequency shift for the KSCN peak at 2050 cm^{-1} (Bellamy, 1975). No significant band broadening or peak shift was observed for this peak in the spectra recorded for humic substances.

To further address this question, the average absolute peak height response (in absorbance units, AU) for the KSCN peak at 2050 cm^{-1} was compared for spectra obtained from both the KHP experiments and humic substance analyses. If part of KSCN were masked by interactions with the humic substances, the mean KSCN response for these analyses would be expected to be lower than the mean for the KHP analyses. The average KHP peak height (0.269 ± 0.085; $n = 13$) was not significantly different ($p < 0.01$) from the humic substance peak heights (0.252 ± 0.115; $n = 71$), although the average peak height for the humic substances is slightly smaller.

The reproduciblity of the deposits obtained from solutions of KSCN and humic substances was investigated by obtaining spectra of replicate deposits of a single humic

substance (Table 3.6). The data obtained for humic substances at both pH 11 and pH 4 indicate that the procedure employed in this research for making cast films of humic substance with KSCN as an internal standard produced IR peak height data with precision on the order of 15 to 20%. Considering the complexity of the systems under investigation and the inherently broad nature of the IR peaks produced by humic substances, the precision of this method appears to be reasonable. The linear relationship between the normalized peak height and concentration for KHP (Figure 3.7), the precision of replicate analyses of humic substances (Table 3.6) and the data presented for the absolute response of KSCN with both KHP and humic substances indicate that this procedure provides a means for the quantitative comparison of the peak intensities between IR spectra obtained for different humic substances.

Typical IR spectra obtained for Santa Fe River DOC at pH 4 and 11 are presented in Figure 3.8. These spectra are comparable to the spectra reported for other humic substances (Piccolo and Stevenson,1976; Stevenson, 1982; Gerasimowicz and Blyer, 1985; MacCarthy and Rice, 1985; Tomikawa, A. and Oba, Y., 1990). There are fewer peaks in the spectrum recorded at pH 11 than at pH 4, because at elevated pH the peak for -COOH shifts to a lower frequency that combines with peaks due to other functional groups. The peak at 2050 cm^{-1} is the internal standard KSCN. As

Table 3.6. Reproducability of peak height ratios of humic carbon to KSCN (mg KSCN·mg humic C[1]) for select humic substances. Relatively low coefficients of variation (C.V.) illustrate good reproducibility of cast film deposits of humic substances.

ORANGE HEIGHTS SOIL [1]					SANTA FE RIVER DOC [2]				
Freq.	Ave.	Std.	Range	C.V.	Freq.	Ave.	Std.	Range	C.V.
3367	0.267	0.032	0.222-0.323	12.0	3135	0.169	0.034	0.131-0.233	20.0
2943	0.145	0.008	0.137-0.158	5.2	3004	0.203	0.036	0.139-0.240	18.0
2866	0.114	0.008	0.103-0.131	7.3	2936	0.219	0.033	0.168-0.255	15.0
1589	1.329	0.263	0.861-1.671	19.8	2552	0.135	0.030	0.102-0.178	21.9
1422	0.953	0.144	0.695-1.181	15.2	1719	0.685	0.117	0.487-0.824	17.1
1026	0.315	0.044	0.253-0.389	14.0	1607	0.300	0.036	0.244-0.336	12.1
					1403	0.295	0.043	0.245-0.360	14.6
					1233	0.415	0.084	0.282-0.491	20.3
					1009	0.384	0.062	0.267-0.462	16.2

[1] pH = 4; N = 6
[2] pH = 11; N = 6

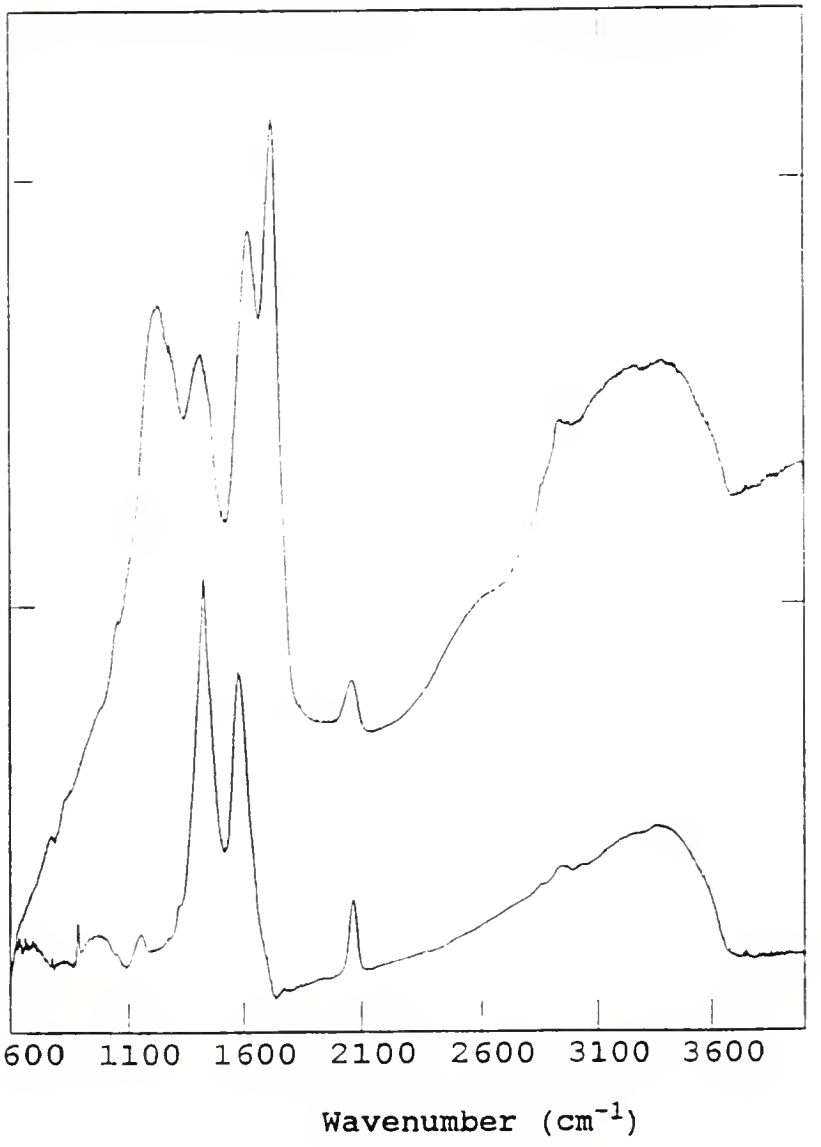

Figure 3.8. Infrared spectra recorded from cast films for the Aldrich Humic Acid sample at pH 11 (lower curve, 12.5 $mg \cdot mL^{-1}$) and pH 4 (upper curve, 10.4 $mg \cdot mL^{-1}$). The peak at 2050 cm^{-1} is the KSCN internal standard peak.

noted earlier, this region of the spectrum is blank for humic substances. The remainder of the peaks in the spectra have been assigned (Table 3.7) based on the assignments previously reported for humic substances (Stevenson, 1982; MacCarthy and Rice, 1985; Thurman , 1985) and other complex molecules (Bellamy, 1975). Although the bands are broad due to the diverse environments for each functional group class within a particular humic source (MacCarthy and Rice, 1985), there is still a large amount of information on the functionality of a humic substance which may be obtained from IR analysis (Schnitzer and Kahn, 1972; Stevenson, 1982).

The major application of IR analyses to humic substances has been the comparison of the relative intensities of different absorption bands within a spectrum of a single humic substance. This approach allows the comparison of the functional groups assigned to these bands within a particular humic substance. The internal standard facilitates quantitative comparison of peak intensities between different humic sources. This allows IR spectra of humic substances to be used for semi-quantitative comparisons of different functional groups within a given humic source as well as across different humic sources. The application of IR spectroscopy with an internal standard is therefore similar to the current use of ^{13}C NMR spectroscopy in the investigation of the functional group composition of

Table 3.7. Infrared spectral adsorption band frequency ranges for functional group components of humic substances.

FREQUENCY (cm^{-1})	FUNTIONAL GROUP
3400-3200	-OH stretch from -COOH and -COH [1,2]
3100-2800	-CH stretch from -CH, $-CH_2$, and $-CH_3$ [1,2]
2700-2400	-OH stretch from strongly H-bonded -COOH [3,4]
1700	-C=O stretch from -COOH [1,2]
1650-1600	aromatic -C=C- vibration; -C=O stretch from H-bonded conjugated ketones; and -COO- asymmetric stretch [1,2]
1400	-OH and -CO deformation from alcohlic and phenolic -OH; and -COO- symmetric stretch [1,2]
1220-1200	-CO stretch and -OH bending from -COOH [1,2]
1050-1020	-CO stretch of poly-saccharides; and Si-O stretch from silicate impurities [1,2]
925	-OH bend; carboxylic acid dimers [5,8]

[1] Stevenson, 1982
[2] MacCarthy and Rice, 1985
[3] Schnitzer and Griffith, 1975
[4] Piccolo and Stevenson, 1982
[5] Bellamy, 1975
[6] Gerasimowiz and Byler 1985

humic substances. ^{13}C NMR spectra are used to quantitatively compare the composition of the various functional groups within and between different sources of humic substances (Wershaw, 1985; Wilson, 1987; Schnitzer, 1991).

The normalized mean peak heights of the humic substances investigated in this study indicate that there are significant differences in their structure and composition of (Tables 3.8 and 3.9 and Appendix 1). The IR spectra of humic substances are, however, complex due to the large number of IR active vibrational and stretching frequencies of the different functional groups present (Table 3.7). Therefore, the structural information available from each spectral band will be discussed in order from high to low frequency.

3400 to 3200 cm^{-1} region. The 3000 cm^{-1} region of the humic substance spectra has been attributed to the -OH stretching of alcohols, phenols and carboxylic acids (Thurman, 1985; Lobartini and Tan, 1988). The band for -OH stretching in simple molecules has been reported to be around 3600 cm^{-1} (Bellamy,1975). However, this band occurs at lower frequencies in humic substances due to H-bonding (Stevenson, 1982). The -OH stretch of strongly H-bonded carboxylic acids occurs in the 2400 to 2700 cm^{-1} spectral region (Schnitzer and Griffith, 1975; Eltantawy and Baverez, 1978).

Table 3.8. Mean infrared absorption peak ratios of humic substance mass to KSCN (mg KSCN·mg humic^{-1}) that are normalized to humic substance mass. Comparative values are reported for analyses made on the humic substances at pH 4 and pH 11.

HUMIC SOURCE	FREQUENCY RANGE (cm^{-1})									
	3400–3200	3000–2900	2900–2800	2700–2400	1700	1650–1600	1400	1220–1200	1050–1000	925
pH 4										
Aldrich Humic Acid	0.223	0.267	0.246	0.148	0.710	0.520	0.237	0.589	0.522	0.399
IHSS Humic Acid	0.364	0.285	0.264	0.148	0.548	0.500	0.311	0.465	0.375	0.215
Pine Mt. Soil	0.108	0.131	0.115	0.109	0.173	0.231	0.178	0.138	0.125	0.080
Orange Hts. Soil	0.163	0.152	0.155	0.103	0.430	0.284	0.231	0.224	0.241	0.188
Newnans Lake Sed. 11	0.265	0.333	0.302	0.173	0.794	0.439	0.342	0.710	0.881	0.583
Newnans Lake Sed. 16	0.277	0.266	0.304	0.190	0.838	0.458	0.329	0.328	0.472	0.300
Newnans Lake Sed. 18	0.329	0.334	0.389	0.218	0.888	0.372	0.298	0.603	0.663	0.439
Santa Fe River DOC	0.169	0.203	0.219	0.125	0.685	0.300	0.295	0.415	0.384	0.258
St. Marys River DOC	0.127	0.150	0.163	0.128	0.510	0.293	0.258	0.315	0.289	0.219
Orange Hts. DOC	0.158	0.166	0.148	0.113	0.448	0.289	0.238	0.282	0.218	0.197
Newnans Lake DOC	0.182	0.247	0.233	0.145	0.709	0.238	0.261	0.360	0.373	0.292

Table 3.8. (continued)

HUMIC SOURCE	FREQUENCY RANGE (cm^{-1})									
	3400–3200	3000–2900	2900–2800	2700–2400	1700	1650–1600	1400	1220–1200	1050–1000	925
Suwannee River FA	0.181	0.201	0.203	0.127	0.519	0.281	0.220	0.231	0.277	0.209
Suwannee River HA	0.142	0.152	0.162	0.145	0.400	0.356	0.234	0.228	0.245	0.177
pH 11										
Aldrich Humic Acid	0.197	0.168	0.142	nd	nd	0.741	0.873	nd	nd	nd
IHSS Humic Acid	0.203	0.181	0.197	nd	nd	0.722	1.997	nd	nd	nd
Pine Mt. Soil	0.185	0.160	0.131	nd	nd	0.463	0.692	nd	nd	nd
Orange Hts. Soil	0.111	0.069	0.048	nd	nd	0.554	0.397	nd	nd	nd
Newnans Lake Sed. 11	0.121	0.102	0.095	nd	nd	0.398	0.275	nd	nd	nd
Newnans Lake Sed. 16	0.137	0.140	0.129	nd	nd	0.388	0.293	nd	nd	nd
Newnans Lake Sed. 18	0.118	0.177	0.145	nd	nd	0.457	0.471	nd	nd	nd
Santa Fe River DOC	0.151	0.113	0.091	nd	nd	0.553	0.570	nd	nd	nd
St. Marys River DOC	0.123	0.128	0.115	nd	nd	0.672	0.565	nd	nd	nd

Table 3.8. (continued)

HUMIC SOURCE	FREQUENCY RANGE (cm^{-1})									
	3400–3200	3000–2900	2900–2800	2700–2400	1700	1650–1600	1400	1220–1200	1050–1000	925
Orange Hts. DOC	0.171	0.153	0.128	nd	nd	0.710	0.594	nd	nd	nd
Newnans Lake DOC	0.147	0.155	0.139	nd	nd	0.595	0.465	nd	nd	nd
Suwannee River FA	0.198	0.214	0.190	nd	nd	0.645	1.984	nd	nd	nd
Suwannee River HA	0.227	0.217	0.223	nd	nd	0.732	1.943	nd	nd	nd

Table 3.9. Mean infrared absorption peak ratios of humic substance carbon mass to KSCN (mg KSCN·mg humic C^{-1}) that are normalized to humic substance carbon mass. Comparative values are reported for analyses made on the humic substances at pH 4 and pH 11.

HUMIC SOURCE	FREQUENCY RANGE (cm^{-1})									
	3400–3200	3000–2900	2900–2800	2700–2400	1700	1650–1600	1400	1220–1200	1050–1000	925
pH 4										
Aldrich Humic Acid	0.439	0.526	0.483	0.291	1.398	1.023	0.467	1.160	1.027	0.785
IHSS Humic Acid	0.633	0.495	0.458	0.257	0.954	0.870	0.541	0.809	0.652	0.373
Pine Mt. Soil	0.184	0.222	0.196	0.185	0.295	0.393	0.302	0.234	0.212	0.137
Orange Hts. Soil	0.341	0.319	0.325	0.215	0.902	0.595	0.484	0.469	0.505	0.395
Newnans Lake Sed. 11	0.575	0.725	0.657	0.377	1.726	0.953	0.744	1.543	1.915	1.266
Newnans Lake Sed. 16	0.682	0.656	0.748	0.468	2.064	1.129	0.811	0.807	1.162	0.738
Newnans Lake Sed. 18	0.779	0.790	0.919	0.516	2.099	0.879	0.704	1.424	1.567	1.039
Santa Fe River DOC	0.342	0.411	0.443	0.252	1.386	0.608	0.597	0.840	0.777	0.522
St. Marys River DOC	0.247	0.292	0.319	0.250	0.996	0.571	0.504	0.615	0.564	0.429
Orange Hts. DOC	0.313	0.328	0.293	0.224	0.886	0.572	0.472	0.559	0.432	0.391
Newnans Lake DOC	0.362	0.492	0.464	0.288	1.411	0.474	0.520	0.716	0.743	0.581

Table 3.9. (continued)

HUMIC SOURCE	FREQUENCY RANGE (cm^{-1})									
	3400–3200	3000–2900	2900–2800	2700–2400	1700	1650–1600	1400	1220–1200	1050–1000	925
Suwannee River FA	0.336	0.373	0.378	0.237	0.965	0.522	0.408	0.430	0.515	0.389
Suwannee River HA	0.262	0.280	0.300	0.267	0.737	0.656	0.432	0.420	0.452	0.327
pH 11										
Aldrich Humic Acid	0.394	0.335	0.284	nd	nd	1.481	1.744	nd	nd	nd
IHSS Humic Acid	0.356	0.318	0.346	nd	nd	1.268	3.504	nd	nd	nd
Pine Mt. Soil	0.315	0.272	0.223	nd	nd	0.788	1.180	nd	nd	nd
Orange Hts. Soil	0.267	0.145	0.114	nd	nd	1.328	0.952	nd	nd	nd
Newnans Lake Sed. 11	0.319	0.268	0.251	nd	nd	1.045	0.722	nd	nd	nd
Newnans Lake Sed. 16	0.460	0.472	0.434	nd	nd	1.308	0.988	nd	nd	nd
Newnans Lake Sed. 18	0.332	0.407	0.407	nd	nd	1.280	1.321	nd	nd	nd
Santa Fe River DOC	0.310	0.231	0.187	nd	nd	1.133	1.168	nd	nd	nd
St. Marys River DOC	0.241	0.251	0.225	nd	nd	1.318	1.107	nd	nd	nd

Table 3.9. (continued)

HUMIC SOURCE	FREQUENCY RANGE (cm^{-1})									
	3400–3200	3000–2900	2900–2800	2700–2400	1700	1650–1600	1400	1220–1200	1050–1000	925
Orange Hts. DOC	0.346	0.310	0.259	nd	nd	1.439	1.205	nd	nd	nd
Newnans Lake DOC	0.314	0.330	0.297	nd	nd	1.269	0.992	nd	nd	nd
Suwannee River FA	0.371	0.402	0.356	nd	nd	1.209	3.718	nd	nd	nd
Suwannee River HA	0.433	0.411	0.424	nd	nd	1.395	3.703	nd	nd	nd

The peak height ratios for the 3300 cm^{-1} region of the spectra were generally higher for each humic substance in the spectra recorded at pH 4 than for this region of the spectra recorded at pH 11 (Tables 3.7 and 3.8). A decrease in the peak intensity in this spectral region would be expected with increased pH due to the ioniation of carboxylic acid hydroxyl group. The loss of the carboxylic acid contribution to the 3400-3200 cm^{-1} peak was also supported by the disappearance of the small peak in the 2400 to 2700 cm^{-1} region (due to strongly H-bonded -OH of -COOH) when the pH is increased from 4 to 11 (Schnitzer and Griffith, 1975). This is consistent with the 3400 to 3200 cm^{-1} band resulting from a combination of alcoholic, phenolic and carboxylic hydroxyl groups at pH 4 and only alcoholic and phenolic hydroxyl groups at pH 11. These results are also in agreement with the reported decreased intensity of the -OH absorption in the 3300 cm^{-1} region when humic substances form metal salts (Piccolo and Stevenson, 1982) and when carboxylic acids react to form esters (Wagner and Stevenson, 1965; Stevenson and Goh, 1972). All of these reactions involve the loss of the carboxyl proton and, therefore, the loss of the -COOH contribution to the absorption of the -OH stretch at 3300 cm^{-1} in the IR spectra.

3100 to 2800 cm^{-1} region. Absorption due to aromatic and aliphatic -CH stretching occurs in the range of 3100 to

2800 cm^{-1} (Bellamy, 1975; Stevenson, 1982). Aromatic -CH stretching occurs at frequencies slightly higher than 3000 cm^{-1}, aliphatic -CH stretching occurs in the 2800 to 2950 cm^{-1} region of the spectrum. These peaks are generally observed as shoulders on the broad -OH absorption band in the IR spectra of humic substances. Aliphatic -CH absorption bands are almost always observed in spectra of humic substances, while the aromatic -CH stretch is rarely reported (MacCarthy and Rice, 1985). This is most likely due to the fact that the -CH stretch for aromatic carbon is much weaker than that for aliphatic carbon (Stevenson, 1982) and the intensity of the aromatic -CH stretch is reduced significantly by substitution on the aromatic ring (Bellamy, 1975). The data for the aliphatic -CH stretching bands (Tables 3.7 and 3.8) indicate the presence of aliphatic structures in the humic substances investigated. No significant absorption was observed for aromatic -CH stretching in this study.

2700 to 2400 cm^{-1} region. The small absorption peak in the 2700 to 2400 cm^{-1} region of the spectrum has been attributed to the -OH stretching of strongly H-bonded carboxylic acids (Bellamy, 1975; Schnitzer and Griffith, 1975; Eltantawy and Baverez, 1978; Piccolo and Stevenson, 1982). Schnitzer and Griffith (1975) reported the loss of this peak from IR spectra when humic substances were titrated base. Similar results were obtained in the current

study for spectra recorded at pH 4 and 11 (Figure 3.8). The low frequency shift of this peak at low pH and its disappearance at high pH support its assignment to the hydroxyl groups of H-bonded carboxylic acids (Bellamy, 1975; Piccolo and Stevenson).

A peak was observed in this region in the spectra recorded at pH 4 for all humic substances investigated (Tables 3.7 and 3.8). The peak was generally the weakest band in each spectra. The exceptions were the Pine Mt. Soil, St. Mary's River DOC and Suwannee River Humic acid samples. These samples had normalized peak intensities in this region that were approximately equal to the intensities of the -OH stretch at 3300 cm^{-1}, suggesting a large portion of the hydroxyl groups in these samples occur in H-bonded carboxyl groups. It should be noted that H-bonding of carboxylic hydroxyl groups would tend to reduce the polarity of these functional groups.

1700 cm^{-1} region. The IR spectra of humic substances recorded at pH 4 were dominated by the presence of a strong absorption band near 1700 cm^{-1}. This band has been assigned to the -C=O stretch of neutral carboxylic acids, although open chain, saturated ketones also absorb in this region (Bellamy, 1975). The 1700 cm^{-1} peak is one of the more easily assigned and least controversial peaks in the IR spectra of humic substances (MacCarthy and Rice, 1985). In addition, the absorptivity of this band has been correlated

with the cation exchange capacity of humic substances (Theng and Posner, 1967; MacCarthy and Rice, 1985). Other studies have reported the loss of this peak when humic substances are titrated from low to high pH (Schnitzer and Griffith, 1975; Piccolo and Stevenson, 1982) or salts are formed with K^+ (Theng and Posner, 1967; Piccolo and Stevenson, 1982).

The IR peak height ratios (Tables 3.7 and 3.8) for the spectra recorded at pH 4 indicate that the -COOH peak at 1700 cm-1 is the largest peak for all humic substances investigated, except for Pine Mt. Soil. The complete absence of this peak in the spectrum recorded at pH 11 for Santa Fe River DOC compared with the spectrum recorded at pH 4 (Figure 3.8) is typical for humic substances investigated in this dissertation research as well as for other humic substances (Vinkler *et al.*, 1976; Piccolo and Stevenson, 1982). This confirms that the peak at 1700 cm^{-1} is the result of -C=O in carboxylic acids. The lack of a significant peak in the 1700 cm^{-1} region at pH 11 for all humic substances in my research is evidence for the low level of saturated, open chain ketonic functional groups (Theng and Posner, 1967). The small shoulder in the vicinity of 1770 cm^{-1} in the spectra of the aquatic humic substances may be due to 5, 6, and 7- membered anhydrides (Stevenson and Goh, 1974). The close proximity of carboxylic acids to one another in humic substances has been

shown to allow the formation of anhydrides which absorb in this spectral region (Wagner and Stevenson, 1965).

1650 to 1600 cm^{-1} region. The absorbance in the 1650 to 1600 cm^{-1} region of the spectrum is more complex than any of the spectral regions discussed thus far. The -C=O stretch of H-bonded, conjugated ketones has been reported to absorb between 1550 to 1650 cm^{-1} (Bellamy, 1975). Quinone absorbs at 1660 cm^{-1}, but the absorption is shifted to lower frequencies with H-bonding (Wagner and Stevenson, 1965). Aromatic -C=C- vibration has been reported to absorb over this entire range (Stevenson, 1982). However, electron withdrawing substituents and asymmetric substitution are known to shift the aromatic -C=C- frequency to near 1600 cm^{-1} (Bellamy, 1975). Considering the degree of substitution proposed for humic substances (Stevenson, 1985), the aromatic carbon vibration would be expected to occur near 1600 cm^{-1} in these compounds (Schnitzer, 1978; Thurman, 1985).

The asymmetric stretch of the carboxylate ion also absorbs near 1600 cm^{-1}. The -C=O stretch for neutral carboxylic acids at 1700 cm^{-1} shifts to around 1600 cm^{-1} when the pH is increased and the $-COO^-$ ion is formed (Schnitzer and Griffith, 1975; Piccolo and Stevenson, 1982). Therefore, the peaks at about 1610 cm^{-1} in the spectra recorded at pH 4 have been assigned to aromatic carbon and possibly H-bonded, conjugated ketones. This region in the

spectra recorded at pH 11 included the contributions of these functional groups plus absorption due to the carboxylate anions (Stevenson, 1982; MacCarthy and Rice, 1985).

The spectra of the humic substances investigated in this dissertation research contained significant peaks in the 1600 to 1650 cm^{-1} region of the spectrum, regardless of the pH (Tables 3.7 and 3.8). The peak was centered at 1610 ± 5 cm^{-1} in the spectra recorded at pH 4, with the exception of the Newnans Lake sediment samples which were closer to 1650 cm^{-1}. Absorption at 1610 cm^{-1} is consistent with an assignment of highly substituted aromatic carbon (Bellamy, 1975). This spectral region at low pH has generally not been attributed to aromatic carbon because of the lack of absorption in the 1500 cm^{-1} region (Theng and Posner, 1967: Eltantawy and Baverez, 1978). Simple aromatic compounds have strong absorption bands at both 1600 and 1500 cm^{-1} (Silverstien and Bassler, 1967; Bellamy, 1975). However, asymmetric substitution of aromatic structures causes an increase in the intensity of the 1600 cm^{-1} absorption and a significant decrease in the 1500 cm^{-1} band (Bellamy, 1975). Therefore, the band in the 1600 cm^{-1} region of the spectra recorded at pH 4 was considered to be mainly due to the aromatic carbon content of the humic substances investigated.

1400 cm^{-1} region. The absorbance in the 1400 cm^{-1} region of the spectrum has also been assigned to more than one functional group. These included the -OH deformation and -CO stretch of alcoholic and phenolic hydroxyl groups and the symmetric stretch of the carboxylate anion (Schnitzer, 1978; Stevenson, 1982; Thurman, 1985). Therefore, the peak near 1400 cm^{-1} can be attributed to alcoholic and phenolic hydroxyl groups at pH 4 but results from the combined absorption of carboxylate anions as well as alcoholic and phenolic hydroxyl groups at pH 11 (Schnitzer and Khan, 1972).

The spectra recorded for the humic substances in this dissertation research at pH 11 and 4 all contained a peak near 1400 cm^{-1} (Tables 3.8 and 3.9). The peak ratios in this spectral region were larger at pH 11 than at pH 4 for all humic substances investigated (Tables 3.8 and 3.9). The peak near 1400 cm^{-1} was often the dominate feature of the spectra of humic substances recorded at pH 11. This is not surprising since the carboxylic acid absorbance at 1700 cm^{-1} at pH 4 is the dominate peak and the peak at 1400 cm^{-1} results in part from the carboxylate anion.

Other spectral regions. The remaining peaks in the IR spectra for the humic substances investigated are the 1200 to 1220 cm^{-1} region assigned to the -CO stretch and -OH deformation of neutral carboxylic acids (MacCarthy and Rice, 1985; Thurman, 1985), the 1020 to 1050 cm^{-1} region assigned

to the -CO stretch of polysaccharides and the -SiO stretch of silicate impurities (Stevenson, 1982; Thurman, 1985) and the -OH bend of carboxylic acid dimmers at 925 cm^{-1} (Bellamy, 1975; Gerasimowiz and Byler, 1985). The assignment of the 1200 to 1220 cm^{-1} band to carboxylic acid is consistent with the loss of this peak from the spectrum when the pH is increased from 4 to 11 (Tables 3.7 and 3.8, also: Schnitzer and Griffith, 1975; Piccolo and Stevenson, 1982). The assignment of the peak in the 1020 to 1050 cm^{-1} region of the spectrum to polysaccharides may not be valid for the Newnans Lake Sediment samples. These samples have a high ash contents (Table 3.1) which are possibly due to silicates. This is consistent with the fact that these samples were obtained from a highly eutrophic lake which contains a large number of diatoms as a silicate source (Gottgens, 1992).

Discussion

The IR spectra of humic substances contain a large amount of information relative to their structure and functionality. The internal standard analysis used in this dissertation research allowed the quantitative comparison of the absorption intensities in different regions of the spectrum among humic substance sources. The usefulness of these data relative to understanding the structure and composition of humic substances was greatly increased by the

ability to quantitatively compare spectra of different humic substances.

The internal standard method also facilitated the comparison of absorption intensities within spectra. For example, a number of absorbances have been assigned to carboxylic acids (Table 3.7): -OH of COOH (3300 cm^{-1}), H-bonded -OH of -COOH (2500 cm^{-1}), -C=O (1700 cm^{-1}), -C-O stretch and -OH bend (1220-1200 cm^{-1}) (Stevenson, 1982; MacCarthy and Rice, 1985) and the -OH bend for carboxylic acid dimmers (925 cm^{-1}) (Bellamy, 1975; Gerasimowiz and Byler, 1985). The assignment of the 925 cm^{-1} band to carboxylic acid dimmers was based on the spectra of pure compounds (Bellamy, 1975) and humic substances (Gerasimowicz and Byler, 1985). The IR spectra of humic substances are not often discussed below 1000 cm^{-1} (Schnitzer and Kahn, 1972; Stevenson, 1982; MacCarthy and Rice, 1985). Therefore, to confirm this assignment, the peak ratio for the 925 cm^{-1} peak was correlated with the peaks ratios obtained for the other carboxylic acid absorptions. The 925 cm^{-1} band correlated well with all the carboxylic acid peaks recorded at pH 4 (Table 3.9): -C=O stretch (1700 cm^{-1}) (r2 = 0.74, $p < 0.01$), -C-O stretch and -OH bend (1220-1200 cm^{-1}) ($r^2 = 0.90$, $p < 0.01$), and -OH stretch of H-bonded -COOH (2500 cm^{-1}) ($r^2 = 0.62$, $p < 0.01$). The lower correlation for the -C=O stretch at 1700 cm^{-1} compared to correlation for the peak at 1220 to 1200 cm^{-1} may reflect

the fact that the former peak may contain absorption due to the -C=O stretch of ketones (Stevenson, 1982; MacCarthy and Rice, 1985). The correlations observed for the peak at 925 cm^{-1} with other carboxylic acid peaks supports the assignment of this peak to carboxylic acids. Further, the correlation between the carboxylic acid dimer peak and the H-bonded carboxylic acid peak indicates that a significant portion of the carboxylic acid dimers are H-bonded (Gerasimowicz and Byler, 1985).

Other correlations observed for the carboxylic acid absorption data are between the H-bonded -OH of COOH (2500 cm^{-1}) and the -C=O of COOH (1700 cm^{-1}) in the spectra recorded at pH 4. The correlation is strong for the data normalized to both grams of humic substance (Table 3.8, $r^2 = 0.66$, $p < 0.01$) and grams of carbon (Table 3.9, $r^2 = 0.83$, $p < 0.01$). This is similar to the relationship discussed above between the H-bonded -OH of COOH (2500 cm^{-1}) with the -OH bend of carboxylic acid dimmers (925 cm^{-1}). The correlation is again strongest for the data normalized on a carbon weight basis (Table 3.9, $r^2 = 0.64$, $p < 0.01$), but is still significant for the data normalized to the weight of humic substances (Table 3.8, $r^2 = 0.50$, $p < 0.01$).

These data indicate that a large portion of the carboxylic acid functional groups in the humic substances investigated in this dissertation are in close proximity and are strongly H-bonded. From 30 to 80% of the carboxylic

acid functional groups in humic substances have been reported to be in positions close enough to form cyclic anhydrides on treatment with acetic anhydride (Wood *et al.*, 1961; Wagner and Stevenson, 1965; Stevenson, 1982). Piccolo and Stevenson (1982) have reported residual uncomplexed carboxylic acid functional groups when humic substances are saturated with Cu^{++} ions. The Cu^{++} binding capacities of the humic substances in this research (Table 3.4) were significantly lower than and not correlated with their total acidities (Table 3.3). The large portion of carboxylic acids that appear to be strongly H-bonded may provide an explanation for the observed Cu^{++} binding and total acidity data. Strongly H-bonded carboxylic acid functional groups in humic substances should be less accessible to Cu^{++} and would have the effect of lowering the polarity of the humic molecules.

The above discussion of the different carboxylic acid absorbances for humic substances emphasizes one of the strong points of IR analysis. In addition to the quantitative aspects of the internal standard method, IR spectra provide qualitative information on the environments of different functional groups. An example of this is the shift to a lower frequency of the strongly H-bonded -OH absorption which allows it to be differentiated from the alcoholic and phenolic -OH absorption.

A correlation was observed between the -OH band at 3300 cm^{-1} and the -OH absorption at 1400 cm^{-1} ($r^2 = 0.67$, $p < 0.01$) for the spectra recorded at pH 4 (Table 3.9). At pH 4, the absorption at 3300 cm^{-1} is due to alcoholic, phenolic and carboxylic acid -OH, while the absorption at 1400 cm^{-1} is due to only alcoholic and phenolic -OH (Stevenson, 1982; MacCarthy and Rice, 1985). This correlation indicates that the absorption at 3300 cm^{-1} is mainly due to alcoholic and phenolic hydroxyl groups. The apparently small contribution of the carboxylic acid hydroxyl groups to the absorption at 3300 cm^{-1} further supports the earlier conclusion that a large portion of these functional groups are strongly hydrogen bonded.

The internal standard IR analysis also allows the IR data to be quantitatively compared with data obtained for humic substances from other types of analysis. The relatively low level of phenolic functional groups in the humic substances currently under investigation can be demonstrated by the correlations observed between the IR peak ratios of various functional groups and the total acidity data (Table 3.3). The total acidity titration measures acidic hydrogen from both carboxylic acids and phenols (Stevenson, 1982; Perdue, 1985). The 1400 cm^{-1} peak at pH 11 (Table 3.9) is due to the combined absorption of phenolic hydroxyl and carboxylate anion functional groups. If phenolic functional groups were a significant

contribution to the hydroxyl groups in the IR spectra, the peak at 1400 cm^{-1} at pH 11 would be expected to correlate well with the total acidity values. The correlation between this peak and total acidity is poor (r^2 = 0.25). However, the correlations between carboxylic acid peaks at pH 4 (Table 3.9) and the total acidity values are much better. The carboxylic acid peak at 1700 cm^{-1} was most strongly correlated with total acidity (r^2 = 0.64, $p < 0.01$). Both the H-bonded -COOH (2500 cm^{-1}) and the dimer -COOH (925 cm^{-1}) showed weaker but significant correlations with the total acidity values (r^2 = 0.50, $p < 0.01$ and r^2 = 0.52, $p < 0.01$, respectively). These correlations indicate that a significant portion the acidity of the humic substances investigated in this research was due to H-bonded and dimer carboxylic acid functional groups. Further, these correlations between other carboxylic acid IR absorption band and the total acidity indicate that the acidity of these humic susbtances is mainly due to carboxylic acid functional groups and not to phenolic functional groups.

The phenolic content of humic substances has been reported to vary widely (Stevenson, 1982) and is often reported to be quite low compared to the carboxylic acid content (Thurman, 1985). The phenolic content of humic substances may be lower than previously believed based on recent ^{13}C NMR analyses (Perdue, 1985). The correlations between total acidity and both the H-bonded and dimer

carboxylic acid IR peaks, coupled with the earlier strong correlation between total acidity and the O/C atomic ratio (Figure 3.3) indicate that the phenolic content of these humic substances is low compared to the carboxylic acid content. Further more, Cu^{++} binding data, viewed in light of the IR data, support the conclusion that a large portion of the carboxylic acid functional groups in the humic substances currently under investigation exist in molecular environments which reduce their acidic character.

There have been numerous conformational structures proposed for humic substances. These include models which describe humic substances in solution as micelles or membrane like bilayers with hydrophilic groups directed outward into the water and hydrophobic groups pointing into the interior (Stevenson, 1982; Wershaw, 1986). Other models consider humic substances in solution to be linear or random coils (Schnitzer, 1991) The high degree of substitution reported for humic substances, especially polar functional groups (Hayes, 1985; Perdue, 1985), have made these models difficult to reconcile. However, if a large portion of the carboxylic acid functional groups is strongly hydrogen bonded and inaccessible to copper binding, this would explain how sterically hindered polar functional groups might exist inside the hydrophobic region of a micelle or membrane bilayer type humic molecule.

Aromatic carbon. The aromatic carbon absorption at 1600 cm^{-1} at pH 4 (Table 3.9) and the E4/E6 ratio (Table 3.2) were found to be inversely related (Figure 3.9; r^2 = 0.64, $p < 0.01$). As noted earlier, the E4/E6 ratio has been used as an indicator for the degree of humification of humic substances (Stevenson, 1982; Thurman, 1985). There have been reports that this ratio is inversely related to the aromatic carbon content of humic substances (Kononova, 1966; Schnitzer and Khan, 1972). However, the most definitive study thus far found a strong positive relationship (r^2 = 0.90, $p < 0.01$) between the E4/E6 ratio and the particle size and molecular weight of the humic substances investigated (Chen *et al.*, 1977). The aromatic carbon content and molecular weight of humic substances are not necessarily totally independent. The relationship illustrated in Figure 3.9 indicates that the E4/E6 ratio is at least partially determined by the aromatic carbon content of humic substances.

Another correlation observed for the IR data was the linear relationship (r^2 = 0.74, $p < 0.01$) between the H/C atomic ratio (Table 3.1) and the combined IR absorption for $-COO^-$ and aromatic carbon (Table 3.9, 1650-1600 cm^{-1}, pH 11). This relationship is similar to that reported earlier between the H/C atomic ratio and the UV absorption at 272 nm (Figure 3.1, r^2 = 0.64, $p < 0.05$). The UV absorbance at 272 nm for humic substances has been attributed to the

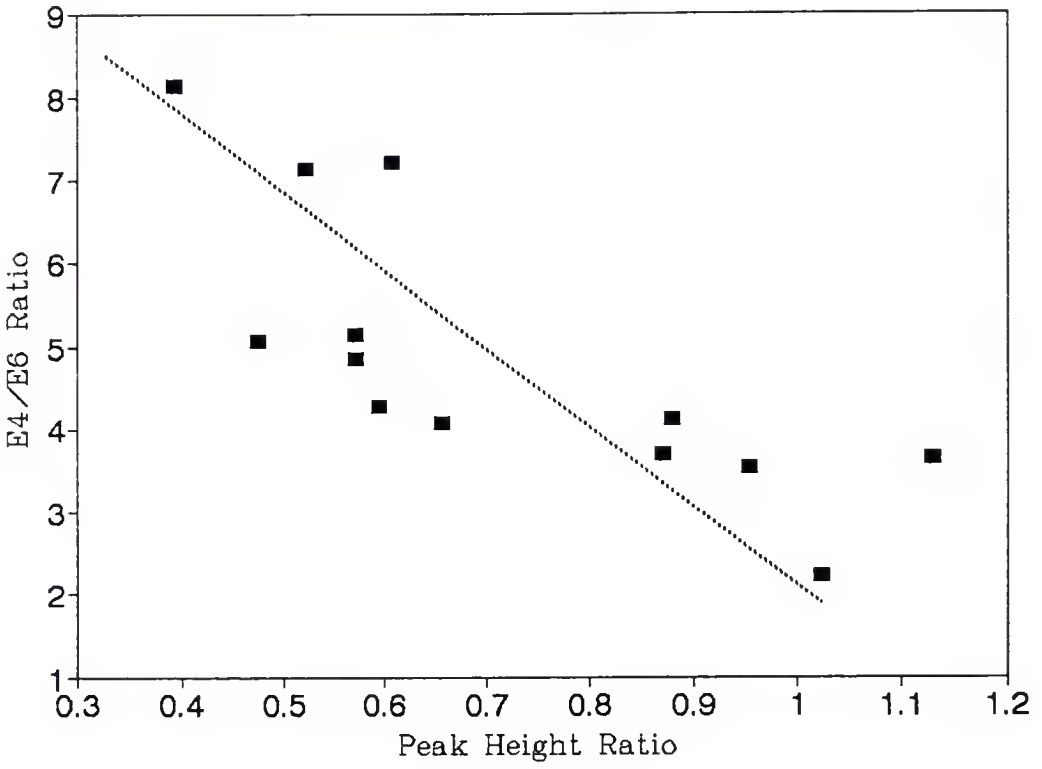

Figure 3.9. The peak height ratio for the humic substance peak at 1600 cm^{-1} to the KSCN peak at 2050 cm^{-1} (g KSCN·g Humic $Carbon^{-1}$) was negatively correlated with the E4/E6 ratio (r^2 = 0.64, $p < 0.01$).

combined absorbance of a variety of functional groups including aromatic carbon and carboxyl groups (Novak *et al.*, 1992). The linear relationship between the combined IR absorption of aromatic and carboxyl functional groups at 1600 cm^{-1} with the H/C atomic ratio further supports the earlier conclusion that this ratio in strongly influenced by both these functional groups.

The relationships discussed above demonstrate the increased utility of internal standard IR analysis over previous qualitative applications of IR spectroscopy for the investigation of the structure and composition of humic substances. The quantitative nature of the data generated by internal standard IR analyses facilitates the direct, quantitative comparison of the functional group composition of different humic substances. The additional qualitative information which can be obtained from IR spectra (ie. the presence of H-bonded -OH of -COOH) makes internal standard IR analysis of humic substances a valuable technique for the investigation of the structure and composition of these complex mixtures.

^{13}C Nuclear Magnetic Resonance Spectroscopy

The application of ^{13}C nuclear magnetic resonance (NMR) spectroscopy to the investigation of the structure and composition of humic substances has occurred relatively recently, beginning in the mid-1970s (Thurman, 1985;

Wershaw, 1985). Investigations of organic molecules using ^{13}C NMR has been facilitated by the development of Fourier transform (FT) NMR instrumentation. Due to the low natural abundance of the ^{13}C isotope, there exists an inherently weak signal for this nucleus. Fourier transform NMR is capable of the relatively rapid acquisition of the large number of spectra required for increased sensitivity using signal averaging (Wilson, 1981). Although a large number of different chemical and spectroscopic techniques have been used to investigate the functional group composition of humic substances, ^{13}C NMR appears to provide more definitive information on the distribution of functional groups than the other techniques (Wershaw, 1985; Wilson, 1987). The ability of ^{13}C NMR to identify and quantify different functional groups in complex molecules has made this technique very attractive for the investigation of the structure and composition of humic substances (Thurman, 1985; Schnitzer, 1991).

Methods

Nuclear magnetic resonance spectroscopy, like other spectroscopic techniques, is dependent on the interaction of molecules, atoms and nuclei with electromagnetic radiation (Wershaw, 1985). Nulcear magnetic resonace spectroscopy is based on the fact that the nuclei of isotopes of some elements (e.g. ^{1}H and ^{13}C) possess magnetogyric or magnetic

spin properties. When ^{13}C nuclei are placed in an externally applied magnetic field, they interact with the applied field by orienting their spin vectors either parallel or anti-parallel to the field. For a given external magnetic field strength, the energy difference between these two states is relatively small for most nuclei. The application of an oscillating radio frequency magnetic field perpendicular to the stationary magnetic field provides enough energy to cause a transition between the lower energy state (parallel) and the higher energy state (anti-parallel). The frequency of the oscillating field that causes this resonance condition is dependent on the magnetic moment of a particular nucleus.

During a NMR experiment, if thermal equilibrium is to be maintained as some nuclei absorb energy and are promoted to the higher energy state, an equal number of nuclei must lose energy and decay to the lower energy state (Wershaw, 1985). This loss of energy, termed relaxation, can occur in two ways; i.e. nuclei interact with the surrounding molecular or solvent matrix (spin-lattice), and/or spin energy is exchanged with other nuclei within a given molecule (spin-spin). The time constants associated with these two relaxation processes are termed T_1 and T_2, respectively. The effects of relaxation on the quantitative application of ^{13}C NMR are important and will be discussed in detail below.

The nucleus in an atom is partially shielded by its electrons from interaction with the externally applied magnetic field. In a molecule, the amount of shielding a nucleus receives from its electrons is dependent on the bonds that the electrons have formed with adjacent atoms. The resonance frequency for a particular nucleus is determined by the effective magnetic field at that nucleus within its particular molecular environment. Thus, different functional groups in organic molecules have different electronic distributions, causing their nuclei to resonate at characteristic frequencies. The displacement of the resonance frequency of a particular nucleus in a molecule from the position for the unshielded atom is referred to as the chemical shift. Chemical shift frequencies are generally reported relative to a standard (often tetramethylsilane, TMS) and are most often reported in parts per million (ppm) (Wershaw, 1985). It is possible to identify a particular functional group based on this resonance frequency shift. The determination of the chemical shift for nuclei is the principal application of NMR for the structural investigations of geochemical samples (Wilson, 1987).

Two types of humic substance samples are routinely analyzed using ^{13}C NMR; liquids and solids. The main advantage of liquid state ^{13}C NMR is that the signals for different functional groups are narrower and the resulting

spectra have better resolution than the spectra obtained for solid state samples (Perdue, 1984; Wilson, 1987). The disadvantage of the liquid state method is that it requires much more time to perform an analysis (≈ 26-30 hrs.) compared with solid state analysis (≈ 3 hrs.) (Perdue, 1984; Schnitzer, 1991). The major problem with solid state ^{13}C NMR is the extremely long spin-lattice relaxation times (T_1) for this nucleus in solids that can cause significant line broadening (Wershaw, 1985). This problem has been overcome by the use of cross-polarization (CP) techniques that allow experiments to be performed based on the fast T1 of the ^{1}H nuclei (Wilson *et al.*, 1984; Wilson, 1987). A detailed discussion of this technique is beyond the scope of this dissertation and the reader is advised to see Wilson (1987) for more detail. Cross-polarization also allows increased sensitivity for ^{13}C nuclei (Newman *et al.*, 1980). Minor line broadening in solid state spectra due to chemical shift anisotropy can be removed by spinning the sample at 54.7° to the stationary magnetic field. This has been termed magic angle spinning (MAS) (Hatcher *et al.*, 1983; Thurman, 1985). The combined techniques of cross-polarization and magic angle spinning for line narrowing is referred to as CP/MAS NMR.

The application of CP/MAS ^{13}C NMR yields relatively high resolution solid state spectra. However, the best solid state spectra of humic substances still show routinely

lower resolution than liquid state spectra (Wershaw, 1985; Wilson, 1987). The broader lines produced in solid state spectra are not a large problem because all the major functional groups are sufficiently resolved (Wershaw, 1985; Wilson, 1987; Schnitzer, 1991). This fact, coupled with the much shorter time required for analysis, led to the choice of solid state ^{13}C NMR for the analysis of functional groups in the humic substances discussed in this dissertation.

Solid state ^{13}C NMR spectroscopy was used for functional group analysis of the humic substances isolated in this research. This method was not used for the three humic substances obtained from the International Humic Substances Society (IHSS) (Table 2.1: Suwannee River Fulvic Acid, Suwannee River Humic Acid and Soil Humic Acid) because quantitative liquid state ^{13}C NMR data were provided by the IHSS (Thorn, 1989; MacCarthy, personal communication).

The ^{13}C NMR spectra were obtained with a Brucker WP-200 SY spectrometer using approximately 300 mg of freeze-dried humic substance as a sample. The spectrometer was operated at 50 MHz for carbon and was equipped with an IBM Solids Control accessory. The spectrometer was externally tuned to the known chemical shifts of t-butyl benzene and the chemical shifts were reported in ppm down field from TMS. Samples were spun at the magic angle (54.7°) at about 4.5 kHz. A cross-polarization contact time of one millisecond

and a repetition rate of 2 seconds were used. For each spectra, 5000 scans were obtained and averaged before Fourier transformation (Antworth *et al.*, 1989).

An internal standard and primary standard were used to quantify the ^{13}C response per unit carbon. The internal standard was an organic polymer, Delrin, with a composition of $H-(CH_2-O)_n-OH$ and the primary standard was para-di-t-butyl benzene (PDBB). Delrin has a single, narrow resonance at 87.2 ppm. This region of the spectrum is of interest in the current investigation; however, the response can be digitally removed from the spectrum before final quantification (Collazo-Lopez, 1989). A machined rod of Delrin was inserted into the center of the sample rotor and the solid sample was packed around it. The response of the Delrin carbon under the NMR experimental conditions was determined by obtaining a spectrum of an accurately weighed PDBB standard and Delrin. Since the amount of carbon in the PDBB standard was known, the response of the Delrin carbon could be calculated by the ratio of the integrated Delrin response to the total spectral response. In this manner, the carbon response observed for any sample could be quantified relative to the Delrin internal standard peak area (Collazo-Lopez, 1989). This internal standard ^{13}C NMR technique was very similar to the internal standard method described earlier for the FTIR analyses.

Results

The data obtained from the ^{13}C NMR analysis indicated differences in the composition of the humic substances investigated (Table 3.10). The spectra recorded for each humic substance analyzed by ^{13}C NMR are contained in Appendix 1 (Figures A1.7-14). Due to limited access to the NMR spectrometer, only a portion of the total number of humic substances obtained during this study were analyzed using this method.

The assignments of the chemical shift regions in the NMR spectra to different functional groups (Table 3.10) are consistent with those reported in the literature (Wilson, 1981; Hatcher *et al.*, 1983; Thurman, 1985; Wershaw, 1985; Newman *et al.*, 1987; Wilson, 1987; Inbar *et al.*, 1989; Thorn, 1989). The chemical shift region from 0 to 50 ppm includes aliphatic carbon bonded to hydrogen (i.e. paraffinic carbon) while the region from 50 to 100 ppm includes aliphatic carbon singly bonded to oxygens. Assignment of the 50 to 100 ppm chemical shift region to carbohydrate is a misnomer because this region includes all aliphatic carbon bonded to hydroxyl and ether groups (Schnitzer and Preston, 1986; Inbar *et al.*, 1989). Also, many authors consider aliphatic carbon to contain the entire chemical shift region from 0 to 100 ppm (Wilson and Goh, 1983; Schnitzer and Preston, 1986; Newman *et al.*, 1987; Lobartini *et al.*, 1991; Schnitzer, 1991). The assignment of

Table 3.10. Functional group percent composition of humic substances as determined by ^{13}C NMR.

HUMIC SOURCE	FUNCTIONAL GROUP (ppm)			
	Aliphatic (0-50)	Carbohydrate (50-100)	Aromatic (100-160)	Carboxyl (160-200)
IHSS Humic Acid	11.5	11.1	47.0	15.3
Pine Mt. Soil	24.1	25.9	37.4	11.1
Orange Hts. Soil	25.7	19.9	23.6	24.4
Santa Fe River DOC	31.4	20.1	17.6	21.3
St. Marys River DOC	25.3	19.7	21.9	21.9
Orange Hts. DOC	36.9	14.2	17.9	19.5
Suwannee River FA	27.0	15.0	28.0	19.0
Suwannee River HA	17.0	12.0	42.0	16.0

100 to 160 ppm to aromatic carbon includes phenolic carbon (140 to 160 ppm) and other aromatic carbon (100 to 135 ppm) (Wershaw, 1985; Newman and Tate, 1991). Finally, the carboxyl chemical shift region from 160 to 200 ppm includes a number of functional groups which contain carbonyl carbons. Aliphatic and aromatic carboxylic acids have chemical shifts of ≈ 180 ppm and 170 to 175 ppm, respectively (Newman *et al.*, 1987). Carbon in ketones and aldehydes appears near 200 ppm (Malcolm, 1990; Thurman, 1985; Wershaw, 1985).

The spectra obtained from the ^{13}C NMR analysis of the humic substances investigated in this study (Appendix 1, Figures A1.7-A1.14) are typical of those which have been reported (Hatcher *et al.*, 1983; Malcolm and MacCarthy, 1986; Schnitzer and Preston, 1986; Wilson, 1987). These spectra contain peaks in all four functional group chemical shift regions of the spectrum (Table 3.10). As is the case with other techniques for investigation of the structure and composition of humic substances,a wide range of functional group compositions have been measured by ^{13}C NMR (Wilson, 1987; Hatcher *et al.*, 1983; Wershaw, 1985). Aromatic carbon content of humic substances measured by solid and liquid state ^{13}C NMR techniques have been reported to range from 10 to 74% of the carbon present in these samples (Wershaw, 1985; Wilson, 1987). The carbohydrate, carboxylate and aliphatic carbon content of humic substances measured by ^{13}C

NMR have also been found to vary (Hatcher *et al.*, 1983; Wilson, 1987; Schnitzer, 1991). In some cases, these compositional variations have been observed to depend on the source of the humic substances (Wilson *et al.*, 1981; Malcolm, 1990). ^{13}C NMR spectra have confirmed that terrestrial humic substances contain more aromatic carbon than freshwater aquatic and marine humic substances (Gillam and Wilson, 1983; Wilson, 1987). Peat derived humic substances have been found to contain higher amounts of oxygen substituted aromatic carbon than other soil derived humic substances (Hatcher *et al.*, 1983).

The solid state ^{13}C NMR spectra recorded for the humic substances in the current study contained the four major peaks normally observed for humic substances (Table 3.10). The spectra indicate that, for those humic substances in the subset that was investigated by ^{13}C NMR, the phenolic carbon peak (140-160 ppm) was generally small, with the exception of the Pine Mt. Soil sample. Two samples, Suwannee River Fulvic Acid and Orange Hts. Soil, showed no phenolic peak at all. The DOC samples, Suwannee River Humic Acid and Soil Humic Acid samples all have peaks in the 140 to 160 phenolic ppm region. However, these peaks were small compared to the peaks for both the other aromatic carbon (100 to 135 ppm) and the carboxyl carbon (160 to 200 ppm). The Pine Mt. Soil sample had a very large phenolic peak, which was a significant part of the overall aromatic carbon content of

this humic substance. This peak was also much larger than the two peaks in the carbonyl region for this humic substance. It should be noted that this sample also indicates the presence of significant ketonic functional groups (≈ 195 ppm) compared to the other carbonyl carbon functional groups in this sample.

The ^{13}C NMR spectra of the Pine Mt. Soil sample is typical of peat humic substances. Hatcher *et al.* (1983) found that peat humic substances contained aromatic structures more highly substituted with oxygen functional groups than other sources of humic substances. The ^{13}C NMR results for the Pine Mt. Soil sample are consistent with the IR data discussed earlier. This sample was found to have a very low carboxylic acid content by both IR and NMR compared with all other samples tested. The high level of phenolic carbon in the Pine Mt. Soil sample could also explain the fact that this sample is an extreme outlier in the correlation between Cu^{++} binding capacity and the H/O atomic ratio (Figure 3.5). The high phenolic content would lead to a high H/O atomic ratio compared with the humic substances that contain larger amounts of carboxylic acid functions.

The apparent low phenolic content observed in the ^{13}C NMR spectra for the other humic substances is consistent with results of the IR analyses and with the literature. The IR data for the carboxylic acid content (1700 cm^{-1}) of these humic substances correlated well with their total

acidity values ($r = 0.80$, $p < 0.01$), indicating that the phenolic content was low compared with the amount of carboxylic acid. A similar relationship was found between the percent carbon for the carbonyl region (160 to 200 ppm) of the ^{13}C NMR spectra and the total acidity (Figure 3.10; $r^2 = 0.96$, $p < 0.01$). These relationships, resulting from two independent measures of the carboxylic acid content of these humic substances, indicate that the majority of their total acidity can be attributed to carboxylic acids. These findings, in combination with the low responses in the 140 to 160 ppm region of the ^{13}C NMR spectra confirm the relatively low level of phenolic functional groups in the humic substances in this dissertation researc

The correlation of the carboxylic acid content of humic substances measured by solid state ^{13}C NMR with total acidity values has been reported by a number of researchers (Hatcher *et al.*, 1981; Schnitzer and Preston, 1986; Lobartini *et al.*, 1991). However, the debate continues over the reason for the difference between the phenolic content of humic substances measured by wet chemical and ^{13}C NMR methods (Perdue, 1985; Lobartini and Tan, 1988; Piccolo *et al.*, 1990). The agreement of carbonyl content measured by ^{13}C NMR and the intensity of the carboxylic acid absorption (1720 cm^{-1}) measured by FTIR has been reported for a number of soil humic substances (Lobartini and Tan, 1988). This is consistent with the correlations discussed

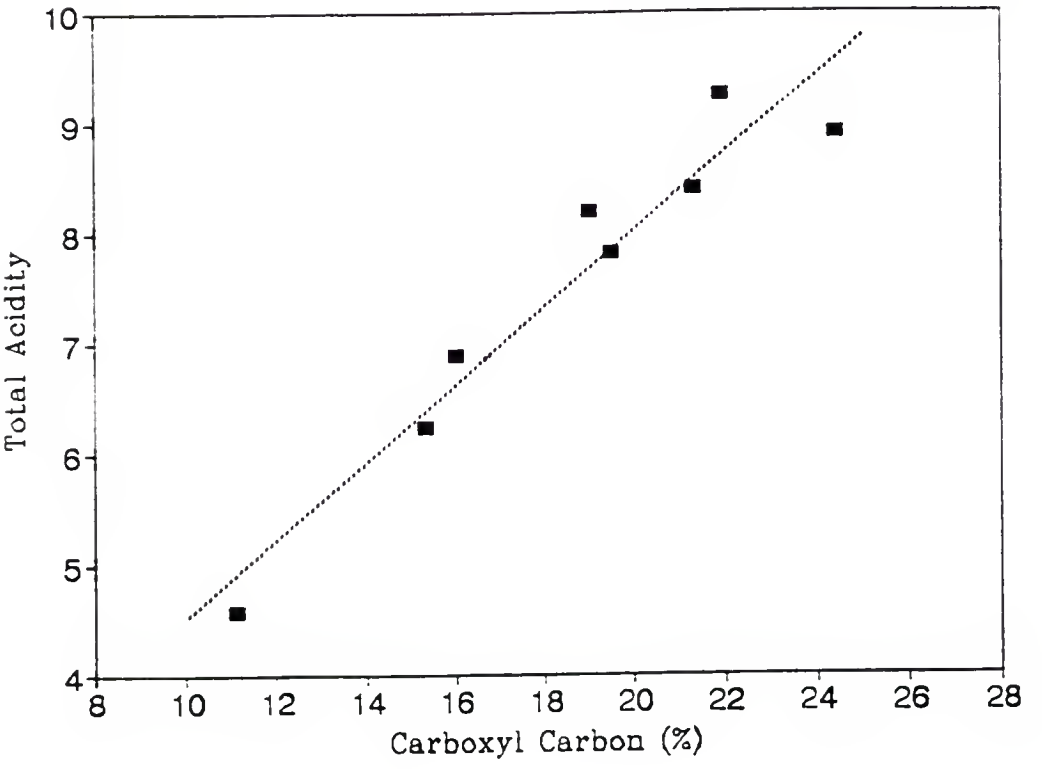

Figure 3.10. The carboxyl carbon content measured by ^{13}C NMR (160-200 ppm) was linearly correlated ($r^2 = 0.92$, $p < 0.01$) with the total acidity (meq $H^+ \cdot g\ Humic^{-1}$) for the humic substances in the current study.

earlier for the total acidity with the carboxylic acid content measured by both ^{13}C NMR (Figure 3.10) and FTIR.

The relationship between total acidity and the carbonyl content of humic substances measured by ^{13}C NMR in the current study is consistent with similar results in the literature. A strong correlation was observed between total acidity and the carboxyl carbon content when the results from this research were combined with the data of Schnitzer and Preston (1986) and Lobartini *et al.* (1991) (Figure 3.11; $r^2 = 0.76$, $p < 0.01$). These data include additional humic substances from both terrestrial (Stevenson and Preston, 1986; Lobartini *et al.*, 1991) and aquatic sources (Lobartini *et al.*, 1991). This correlation supports the conclusion of other researchers that the phenolic content of humic substances may be lower than previously believed based on wet chemical procedures (Hatcher *et al.*, 1981; Hatcher *et al.*, 1983; Perdue, 1985).

The phenolic content of humic substances has been reported to be highly variable (Stevenson, 1982). The amount of acidity due to phenolic functional groups was found to be small compared to that due to carboxylic acid functional groups in a number of humic substances analyzed by ^{13}C NMR (Mikita *et al.*, 1981; Thurman, 1985). Schnitzer and Preston (1986) have suggested a down field movement of the chemical shift for phenolic carbon to the carboxyl chemical shift region in the ^{13}C NMR spectra of humic

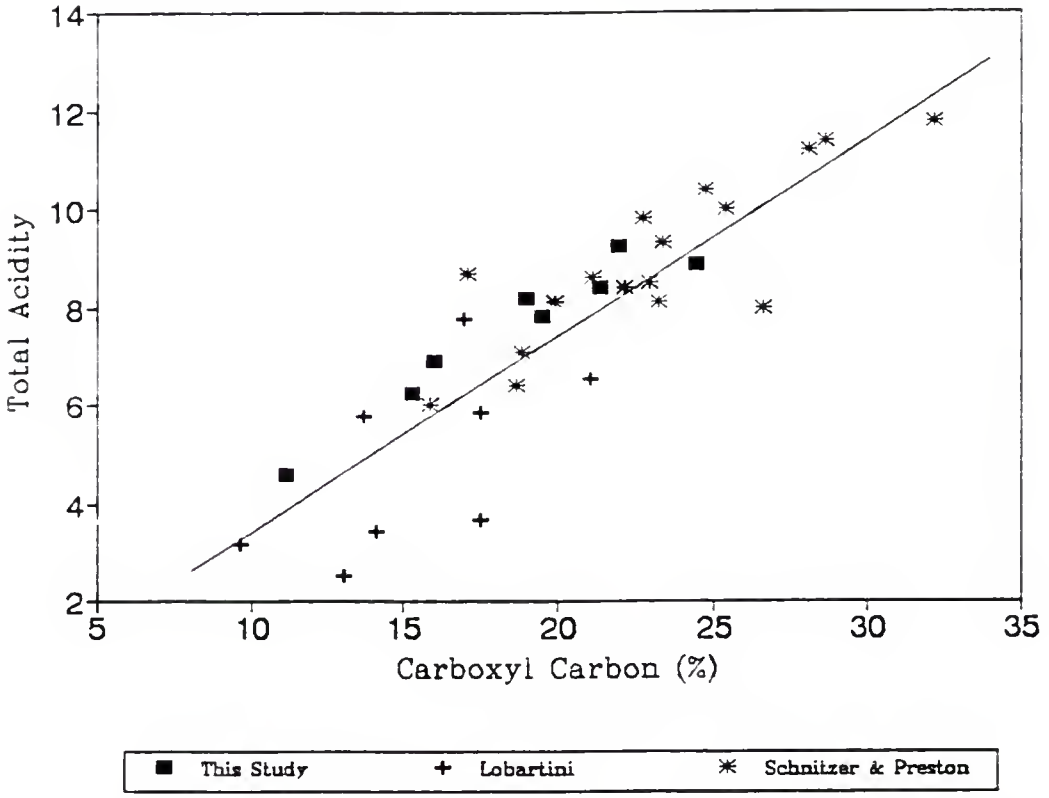

Figure 3.11. A linear relationship between carboxyl carbon content measured by ^{13}C NMR (160-200 ppm) and the total acidity (meq $H^{+} \cdot g$ $Humic^{-1}$) (r^2 = 0.76, $p < 0.01$) was observed for the combined data sets of this study, Schnitzer and Preston (1986), and Lobartini et al. (1991). Sources included both terrestrial and aquatic humic substances.

substances. This proposed movement was evoked to explain the low levels of phenolic carbon detected by this technique compared with the phenolic content calculated using titration methods. The change in phenolic chemical shifts in the ^{13}C NMR spectra of humic substances proposed by Schnitzer and Preston (1986) was based on the observed chemical shifts of highly substituted phenols in simple molecules. However, the degree of substitution required to produce this down field shift is not likely to occur in humic substances (Perdue, 1984). An extensive investigation of a large number of aquatic humic substances using oxidation methods found only one phenolic hydroxyl group for every 13.5 aromatic carbons (Liao *et al.*, 1982).

The calculation of phenol acidity of humic substances based on titration data has been questioned (Stevenson, 1982; Perdue, 1985; Bonn and Fish, 1991). The method is based on the measurement of the total acidity using the $Ba(OH)_2$ titration and the measurement of the carboxylic acid content using calcium acetate titration (Schnitzer, 1972; Stevenson, 1982). Phenolic acidity is calculated as the difference between the total acidity and the carboxylic acid acidity. The carboxylic acid content of humic acids measured using calcium acetate titration are generally considered an operationally defined value and most likely provides underestimates of the true carboxylic acid content (Perdue, 1985; Bonn and Fisher, 1991). The underestimation

of carboxylic acid would lead to an overestimation of the phenolic content based on titration data. The correlations observed for the current study between the total acidity and the carboxylic acid content measured by both ^{13}C NMR and FTIR support the conclusion that the phenolic content of humic substances may be lower than previously believed based on wet chemical methods.

Another functional group that is invariably mentioned as a component of humic substances is ketonic carbonyl (Schnitzer, 1978; Stevenson, 1982; Thurman, 1985). The amount of this functional group present in humic substances has also been disputed. Similar to the measurement of phenolic carbon content, ^{13}C NMR yields lower amounts of ketonic carbon than derivatization/titration methods. The derivatization procedure involves the formation of an oxime which may create ketonic functional groups from reactive acetals (Thurman, 1985). The fact that the amount of oxygen present measured by ^{13}C NMR for humic substances agrees with elemental analysis indicates that the results of ^{13}C NMR are probably valid (Thurman, 1985). The ketonic carbonyl content of humic substances measured by chemical methods has been reported to be as high as 23% (Schnitzer and Khan, 1972), while ^{13}C NMR data have generally found significantly lower amounts of this functional group. Numerous ^{13}C NMR studies have found very little or no ketonic carbon present in the humic substances investigated (Newman *et al.*, 1980;

Wilson *et al.*, 1981; Newman *et al.*, 1987; Malcolm, 1990; Newman and Tate, 1991). Malcolm (1990) reported very weak ^{13}C NMR peaks for ketonic carbon (195 to 210 ppm) in stream fulvic acids, weaker peaks in soil fulvic acids and no ketonic peak in marine fulvic acids. Another report indicated ketonic carbon at less than 1% for nine different soil humic acids (Newman and Tate, 1991). No ketonic peak was observed for humic substances from freshwater (Wilson *et al.*, 1981) or soil (Newman *et al.*, 1980; Newman *et al.*, 1987). These finding are consistent with the ^{13}C NMR data collected in this dissertation research. There are no significant peaks for ketonic carbon in any of the samples investigated, except for the Pine Mt. Soil sample (Appendix 1, Figures A1.7 to A1.14). The low level of ketonic functional groups detected by ^{13}C NMR in the humic substances in this research are important relative to the interpretation of the FTIR data. The peaks in the 1600 to 1650 cm^{-1} region of the spectrum were assigned to a combination of aromatic carbon, carboxylate anion and ketonic carbon (Table 3.7). However, in the spectra recorded at pH 4, there should be very little contribution from carboxylate anion. The ^{13}C NMR spectra indicate that there is no ketonic carbon in these humic substances, except for the Pine Mt. Soil sample. This leads to the conclusion that the IR absorption in the 1600 cm^{-1} region of the

spectra at pH 4, for these humic substances, is due primarily to aromatic carbon.

There was no correlation observed for the current study between the aromatic carbon content measured by ^{13}C NMR (Table 3.10; 100 to 160 ppm) and FTIR (Table 3.9; 1650-1600 cm^{-1}). This may be due to problems associated with measuring aromatic carbon using ^{13}C NMR. Inaccurate measurement of aromatic carbon by solid state CP/MAS ^{13}C NMR can result from the long T_1 relaxation times for highly substituted aromatic carbon (Wilson, 1987). As mentioned earlier, cross polarization allows the ^{13}C nuclei to relax at the much faster rate of the ^{1}H nuclei. Investigations of highly aromatic compounds have revealed that, for carbon which is more than four bonds removed from a proton, the cross polarization mechanism will not allow the ^{13}C to relax rapidly enough for it to be observed in the CP/MAS NMR experiment (Wilson *et al.*, 1984).

A number of authors have investigated the cross polarization contact times and repetition rates necessary to obtain quantitative results for a variety of humic substances. The 1 msec cross polarization time and 2 sec repetition rate used in the current investigation should be sufficient to obtain quantitative ^{13}C NMR spectra (Wilson *et al.*, 1981; Hatcher *et al.*, 1983; Newman *et al.*, 1987; Wilson, 1987). It is not possible to be sure that all aromatic carbon was measured without conducting cross

polarization contact time experiments on each humic substance (Wilson, 1987). Therefore, it is not possible to reconcile the difference between the relative aromatic carbon contents measured by ^{13}C NMR and FTIR.

Traina *et al.* (1990) reported a strong correlation ($r^2 = 0.88$, $p < 0.01$) between the aromatic carbon content of 11 soil humic substances and their absorptivity at 272 nm. However, when this data set was expanded to include 18 additional aquatic and terrestrial humic substances the strength of the correlation decreased ($r^2 = 0.36$) (Novak *et al.*, 1992). The data from Gauthier *et al.* (1987), Traina *et al.* (1990) and Novak *et al.* (1992) have been combined with the data for this resreach to yields a correlation that is intermediate in strength (Figure 3.12; $r^2 = 0.52$, $p < 0.01$) compared to those of Traine *et al.* (1990) and Novak *et al.* (1992). This relationship (Figure 3.12) indicates that the aromatic carbon content of these humic substances accounts for about 50% of the variation in the absorptivity at 272 nm. This correlation is similar to the relationship between the absorptivity at 272 nm and the H/C ratio (Figure 3.1; $r^2 = 0.64$, $p < 0.01$) for the same combined data sets. However, the H/C atomic ratio is a composite of the aromatic, carboxylate and aliphatic carbon content of the humic substances, while both aromatic and carboxylate functional groups contribute to the absorptivity at 272 nm (Gauthier, 1987; Traina *et al.*, 1990). The relationship

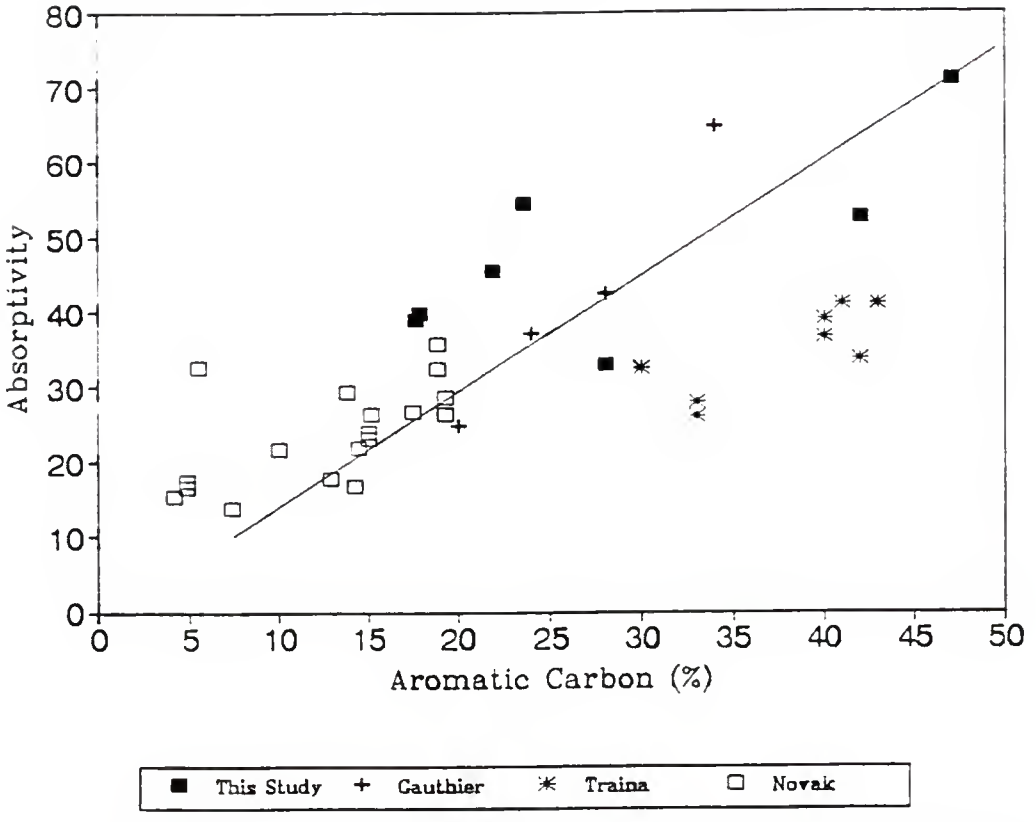

Figure 3.12. A correlation was observed between the aromatic carbon content determined by ^{13}C NMR (100-160 ppm) and the absorptivity at 272 nm ($L \cdot g\ Carbon^{-1} \cdot cm^{-1}$) ($r^2 = 0.52$, $p < 0.01$) for the combined results of this study, Gauthier et al. (1987), Traina et al. (1990), and Novak et al. (1992). A similar relationship was observed for the same combined data set between the H/C atomic ratio and the absorptivity at 272 nm (Figure 3.1, $r^2 = 0.64$, $p < 0.01$).

between aromatic carbon measured by ^{13}C NMR and the absorptivity at 272 nm indicates that the absorptivity at 272 nm is the result of additional functional groups besides aromatic carbon.

These relationships typify the complex nature of humic substances themselves and the difficulty in illuminating their composition and structure. The problems associated with the chemical derivatization/titration method for the measurement of the ketonic carbonyl content illustrate the fact that the process of investigating humic substances often alters them (Thurman, 1985). This is also true of the oxidation and hydrolysis processes that can occur during extraction (Aiken, 1985). Non-destructive techniques such as NMR and FTIR offer potentially powerful techniques for investigations of the structure and composition of humic substances. There are problems and limitations with any technique. However, ^{13}C NMR will no doubt continue to contribute significantly to our understanding of the structure and composition of these extremely complex mixtures.

Summary

1. The elemental compositions of the humic substances isolated in this dissertation research (Table 3.1) are typical of those reported for humic substances obtained from different sources. The inorganic content (ash) of these

humic substances was low, except for the Orange Heights Soil and Newnans Lake Sediment samples.

2. The H/C atomic ratio correlated with both the absorptivity at 272 nm (Figure 3.1; $r^2 = 0.64$, $p < 0.01$) and the E4/E6 ratio (Figure 3.2; $r^2 = 0.50$, $p < 0.01$) when the results from the current study were combined with results from the literature (Nissenbaum and Kaplan, 1972; Ertel and Hedges, 1983; Gauthier *et al.*, 1987; Traina *et al.*, 1990).

3. The E4/E6 ratio obtained for the aquatic humic substances isolated in this study (Table 3.2) were in the range reported for fulvic acids, while those measured for the soil and sediment isolates were typical of humic acids, with the exception of the Pine Mt. Soil sample.

4. Total acidity values measured by $Ba(OH)_2$ (Table 3.3) correlated with the O/C atomic ratio (Figure 3.3; $r^2 = 0.83$, $p < 0.01$) for the humic substances in this study.

5. The copper binding capacities of the humic substances in this research correlated with the H/O atomic ratio (Figure 3.5; $r^2 = 0.88$, $p < 0.01$) but did not correlate with the total acidities.

6. An internal standard IR method was developed which allowed direct quantitative comparison of the functional group compositions of the humic substances in the current study.

7. IR spectra revealed that a significant portion of the carboxylic acid functional groups was strongly hydrogen bonded in the humic substances investigated.

8. The intensities of the IR peak at 1600 cm^{-1} was inversely related to the E4/E6 ratio for the humic substances investigated in the current study (Figure 3.9; $r^2 = 0.64$, $p < 0.01$).

9. Correlations were found between the total acidity and the intensities of the IR bands assigned to carboxylic acid functional groups in a number of different molecular environments (Table 3.9; -COOH, 1700 cm^{-1}, $r^2 = 0.64$, $p < 0.01$; H-bonded -COOH, 2500 cm-1, $r^2 = 0.50$, $p < 0.01$; and dimmer -COOH, 925 cm^{-1}, $r^2 = 0.52$, $p < 0.01$).

10. The ^{13}C NMR spectra of the humic substances in this dissretation research correlated with the total acidity (Figure 3.10; $r^2 = 0.92$, $p < 0.01$). The correlation of these two parameters remained strong when the results of this research were combined with the data of Schnitzer and Preston (1986) and Traina *et al.* (1990) (Figure 3.11; $r^2 = 0.76$, $p < 0.01$).

11. Absorptivity at 272 nm correlated with the aromatic carbon content of humic substances (Figure 3.12;

$r^2 = 0.52$; $p < 0.01$) when the data from the current study were combined with that of other investigations (Gauthier *et al.*, 1987; Traina *et al.*, 1990; Novak *et al.*, 1992). This

data set included 37 humic substances from terrestrial, sedimentary and both freshwater and marine aquatic sources.

Conclusions

1. The different analytical techniques that have been used to investigate the humic substances in this dissertation research have revealed that they are complex mixtures which contain relatively large amounts of oxygen containing functional groups.

2. The information obtained for each analytical procedure is an average for all molecules and functional groups present in the humic substances.

3. Although the elemental compositions of the different humic substances are similar, their functional group compositions are quite different.

4. Based on these data, the different sources of humic substances appear to have compositions that are comparable to humic substances from similar sources reported in the literature.

5. The combination of a number of different analyses yields information on the possible structure of the humic substances.

6. The H/C atomic ratio, absorptivity at 272 nm and E4/E6 ratio are the result of the combined response of multiple functional groups. Both aromatic carbon and

carboxylic acid functional groups contribute to the response observed for these three parameters.

7. The data obtained from both ^{13}C NMR and FTIR spectroscopy combined with the data for total acidity indicate that the oxygenated functional groups of these humic substances are dominated by carboxylic acids.

8. The lack of correlation between humic substance Cu^{++} binding and total acidity indicates that not all the carboxylic acids are available for metal binding.

9. These findings, in combination with the large portion of the carboxylic acids which appear by FTIR to be strongly hydrogen bonded, support a micelle or membrane bilayer model for humic substances in solution.

CHAPTER 4
INTERACTIONS OF HYDROPHOBIC ORGANIC COMPOUNDS WITH DISSOLVED HUMIC SUBSTANCES

Introduction

The interactions between dissolved humic substances and hydrophobic organic compounds (HOCs) have been the subject of great interest since Wershaw *et al.* (1969) first reported increased solubilization of DDT by sodium humate solutions. The increased apparent aqueous solubility of highly insoluble organic compounds in the presence of natural dissolved organic carbon has significant environmental implications. These include reduced partitioning of HOCs from water into suspended solids, sediments and soils (Hassett and Anderson, 1982; Gschwend and Wu, 1985), facilitated transport of these compounds in hydrodynamic systems (Enfield *et al.*, 1989; Backhus and Gschwend, 1990), reduced volatilization rates from water (Mackay *et al.*, 1979), reduced extraction efficiencies for some organic pollutants (Pankow *et al.*, 1988; Maguire and Tkacz, 1989) and decreased uptake and toxicity of HOCs to aquatic biota (McCarthy, 1983; Bitton *et al.*, 1986; Landrum *et al.*, 1987).

Efforts to predict the interactions between HOCs and different sources of dissolved humic substances have been

unsuccessful. Attempts to extend models of HOC interactions with organic carbon in soils and sediments to dissolved humic substances have failed because of the large variation in K_{doc} values observed for a particular HOC with different sources of dissolved humic substances (Whitehouse, 1985; Chiou *et al.*, 1986; Evans, 1988). This observed variation has been postulated to be due to the structure and composition of the dissolved humic substances (Whitehouse, 1985; Chiou *et al.*, 1986; Gauthier *et al.*, 1987; Kile and Chiou, 1989; McCarthy *et al.*, 1989).

To investigate the relationships between the structure and composition of humic substances and their binding of HOCs, sorption experiments were performed using two homologous series of HOCs and the thirteen humic substances characterized in Chapter 3. The K_{doc} data obtained from these studies were used to examine the influence of humic substance structure and composition on these interactions. The differences between the K_{doc} values for the two homologous series of HOCs with a particular humic substance were used to elucidate the mechanism of this sorption process.

Methods

The sorption of HOCs to soils and sediments has been investigated using batch isotherms (Khan, 1978; Stevenson, 1982). The batch isotherm method is based on allowing an

aqueous solution of the HOC to equilibrate with the soil or sediment and then measuring the concentration of the HOC remaining in solution. The experimental design required to assess the interactions of dissolved humic substances with HOCs is more complex than that of the batch isotherm. Separation of the truly dissolved solute (HOC) from the solute associated with the sorbent (dissolved humic substances) in these experiments is more difficult. A number of methods have been used to measure the sorption of HOCs by dissolved humic substances. These included equilibrium dialysis (Carter and Suffet, 1982), fluorescence quenching (Gauthier *et al.*, 1986), differential solubilization (Choiu *et al.*, 1986) gas-phase partitioning (Garbarini and Lion, 1985; Hassett and Milicic, 1985) and reversed-phase chromatography (Landrum *et al.*, 1984).

The gas-phase partitioning and fluorescence quenching techniques are limited in their application to HOCs which have appreciable vapor pressures and those which naturally fluoresce, respectively. Some HOCs investigated using equilibrium dialysis have been found to adsorb to or diffuse too slowly through the dialysis membrane (Carter and Suffet, 1983; Landrum *et al.*, 1984; Yin and Hassett, 1986). The reversed-phase chromatographic method has been reported to be more precise than equilibrium dialysis and is applicable to any compound which is sufficiently hydrophobic to be retained by an octadecyl reverse-phase (C18) column (Landrum

et al., 1984). The C18 column technique is also rapid and inexpensive (Landrum *et al.*, 1984). Therefore, C18 column chromatography was selected for the current study as the method for measuring the partitioning of HOCs between water and dissolved humic substances.

The C18 column method is based on the observation that humic substances are too hydrophilic to be retained by a C18 column (Landrum *et al.*, 1984). When an aqueous solution of a dissolved humic substance and a HOC is passed through a C18 column, the truly dissolved HOC is retained. However, the humic substance and the humic substance/HOC complexes pass through the column unretained. The retained HOC can then be eluted from the C18 column with a non-polar solvent and quantified. The amount of the HOC associated with the humic substances is calculated by subtracting the truly dissolved HOC concentration from the known total concentration of HOC added to the solution (Landrum *et al.*, 1984). The level of partitioning is described by the partition coefficient (K_{doc}) which is normalized to the organic carbon content of the humic substance. The partition coefficient is calculated as follows (Carter and Suffet, 1983):

$$K_{doc} = (\text{g HOC/g humic carbon})/(\text{g HOC/mL water}) \qquad (4.1)$$

The truly dissolved HOC concentration measured by the C18 column technique is the denominator and the amount of HOC associated with the dissolved humic substance is the numerator in Equation 4.1.

To examine the relationship between K_{doc} and the structure and composition of humic substances, the bias between K_{doc} measurements for different humic substances had to be minimized. The reported variation in measured K_{doc} values for a particular HOC with a single humic substance due to experimental conditions (such as pH and ionic strength) led to the adoption of a standard set of conditions in the current research. The rationale was that under constant experimental conditions, any bias would be uniform. K_{doc} measurements were made at pH 7, an ionic strength of 0.001 M NaCl and a humic substance concentration of approximately 20 mg/L.

Solutions of humic substances for K_{doc} measurement were prepared by dissolving freeze-dried humic substances (20 ± 10 mg/L) in 0.001 M NaCl. The pH of the humic solutions was adjusted to 7 using 0.01 M HCl or NaOH. Humic solutions were placed in 100 mL volumetric flask with ground glass stoppers. Sorption of two different series of HOCs by humic substances were investigated: polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs).

The HOCs used in these investigations and their sources are listed in Table 4.1. The numbering system for the

Table 4.1. Sources of hydrophobic organic compounds used in sorption experiments with selected humic substances. The numbering of PCB congeners follows that of Zell and Ballschmitter, (1980).

Compound	Source
PCB 47	Supelco Inc., PCB Mix (No. 4-8738M)
PCB 77	Chemical Services, Inc., (No. 7835G)
PCB 98	Supelco Inc., PCB Mix (No. 4-8738M)
PCB 101	Chemical Services, Inc., (No. 6789G)
PCB 114	Chemical Services, Inc., (No. 6794G)
PCB 126	Chemical Services, Inc., (No. 6796G)
PCB 153	Chemical Services, Inc., (No. 5019G)
PCB 154	Supelco Inc., PCB Mix (No. 4-8738M)
PCB 171	Supelco Inc., PCB Mix (No. 4-8738M)
PCB 200	Supelco Inc., PCB Mix (No. 4-8738M)
Phenanthrene	Supelco Inc., (No. 4-8569M)
Anthracene	Supelco Inc., (No. 4-8567M)
Pyrene	Supelco Inc., (No. 4-8570M)
Chrysene	Supelco Inc., (No. 4-8565M)
Benzo(a)pyrene	Supelco Inc., (No. 4-8564M)
Dibenz(a,h)anthracene	Supelco Inc., (No. 4-8574M)
Benzo(k)fluoranthene	Supelco Inc., (No. 4-9492M)

PCB congeners follows that of Zell and Ballschmitter (1980). Hydrophobic organic compounds were purchased as premixed concentrates (PCB congeners 47, 98, 154, 171 and 200) or as individual pure compounds (additional PCB congeners and PAHs). Working solutions were prepared by serial dilution of premixed concentrates or from stock standard solutions prepared by diluting measured quantities of primary standards into either acetone (PCBs) or an equal volume mixture of methanol/dichloromethane (PAHs).

All HOCs used were spiked into humic substance solutions to yield total HOC concentrations less than or equal to 50% of their aqueous solubilities (Miller *et al.*, 1985; Shaul, 1991). The humic substance/HOC solutions were immediately wrapped in aluminum foil and placed inside a darkened container to protect the humic substances from possible photodegradation. Duplicate solutions were prepared from each humic substance source for K_{doc} measurement with all HOCs. The humic substance/HOC solutions were mixed on a surface shaker overnight and allowed an equilibration time of 24 hours. The solutions appeared to reach equilibrium in this amount of time based on experiments which indicated no increase in K_{doc} values measured when samples were equilibrated from 24 and 72 hours. Typical equilibration times reported in the literature are between 14 and 24 hours (Landrum *et al.*, 1984; McCarthy and Jimenez, 1985; Gauthier *et al.*, 1986; Yin

And Hassett, 1986). However, fluorescence quenching methods applied to PAHs have reported equilibration with dissolved humic substances in less than an hour (McCarthy and Jimenez, 1985; Gauthier *et al.*, 1986; Backhus and Gschwend, 1990). Each batch of samples analyzed by this procedure included a blank and a spike sample. The blank consisted of 0.001 M NaCl and the spike was 0.001 M NaCl which received the same level of HOCs as the humic substance/HOC samples. These samples were also adjusted to pH 7 as needed.

The C18 column technique was applied in the current investigation using 1 g C18 solid phase extraction columns (Fisher Scientific Co., Cat. no. P479). The columns were prewashed to remove any interfering compounds with three 15 mL volumes of DI water, three 15 mL volumes of methanol (Fisher Scientific, Co., Optima Grade, Cat. No. A454-1) and five 15 mL volumes of DI water. The C18 column procedure was run in batch using a manifold (Supelco, Inc., Cat no. 5-7044M) at reduced pressure to facilitate column flow. The manifold was designed with needle valves for flow control of each column. Before each experiment, the flow rate was set to 12 mL/min for each column and the humic substance/HOC solutions were eluted. The flow rate minimized the potential dissociation of the humic substance/HOC complex during the C18 column step (Landrum *et al.*, 1984). After the humic substance/HOC solutions had been passed through the C18 columns, they were dried under vacuum for 30 min. to

remove entrained water. The truly dissolved HOCs which had been adsorbed from the solution were then eluted from the C18 columns using either 10 mL of hexane (Fisher Scientific, Co., Optima Grade, Cat. no. H303-1) for PCBs or 10 mL of 50/50 methanol/dichloromethane (Fisher Scientific, Co., Optima Grade, Cat. nos. A454-1 and D151-1, respectively) for PAHs. These column eluates were concentrated to a final volume of 1.0 mL (PCBs) or 0.5 mL (PAHs) under a stream of dry nitrogen gas using a N-Evap Analytical Concentrator (Organomation, Inc.).

The volumes of humic substance/HOC solutions eluted through the C18 columns were determined by the sensitivities of the procedures used to quantify the HOCs sample concentrates. The PCBs required 20 mL and the PAHs required 50 mL of the humic substance/HOC solutions be eluted to obtain the necessary concentrations of analytes in the final sample concentrates. PCBs were quantified by gas chromatography (GC) and the PAHs were quantified by high performance liquid chromatography (HPLC). Regardless of the technique used to quantify the C18 column concentrates, each sample concentrate was quantified in duplicate.

The C18 column sample concentrates containing PCBs were analyzed using a Varian 3700 gas chromatograph (Varian Associates, Inc.) equipped with a ^{63}Ni electron capture detector (ECD). Individual PCB congeners were separated using a 30 m, DB5 capillary column with a 0.53 mm internal

diameter and 1 μm film thickness (J&W Scientific, Cat. no. 1255032). The column injector was held at 200° C and the detector was held at 300° C. The column was temperature programmed as follows: 5 min. at 120° C, then the temperature was increased at a rate of 5° C/min. to 230° C and held at this temperature for 4 min.. The chromatographic peaks from the ECD were integrated using an HP Model 3390A recording integrator (Hewlett Packard, Inc.).

The GC/ECD system was calibrated by analysis of solutions of PCB congeners at five known concentrations bracketing the concentration range of interest. All PCB calibration curves were linear with intercepts near zero ($r^2 \geq 0.95$). The GC/ECD calibration was checked each day prior to analysis. Peak areas for the PCB congeners obtained from the analysis C18 column concentrates were entered into a Lotus 123 spreadsheet and quantified using the calibration curves generated for the GC/ECD system. The concentrations of the individual PCB congeners truly dissolved in the humic substance/PCB solutions were calculated based on the concentrations of the congeners measured in the C18 column concentrates.

The PAHs were quantified using a modular HPLC system. The solvent was delivered by an Eldex Model 9600 gradient forming HPLC pump operated at a flow rate of 2.0 mL/min. The samples were eluted through a 15 cm long, 4.6 mm ID column packed with 5 μm diameter LCPAH packing material

(Supelco, Inc., Cat. no. 5-8318). The individual PAHs were separated on column using a gradient of acetonitrile (ACN, J.T. Baker, Inc., HPLC Grade, Cat. no. 9017) and water (J.T. Baker, Inc., HPLC Grade, Cat. no. 4218). The solvent gradient was as follows: isocratic at 35% ACN for 2 min., then increase ACN at 1.5%/min to 50% ACN at 12 min., isocratic at 50% ACN to 14 min., then increase ACN at 7.5%/min. to 95% ACN at 20 min., isocratic at 95% ACN to 26 min.. The column was then returned to 35% ACN and allowed to equilibrate at this solvent composition for 5 min. before the next sample was analyzed. The C18 column concentrates and calibration standards were injected (40 μL) using a Wisp Automatic Injector (Waters Associates, Inc.). The PAHs in the column effluent were detected by a Waters Model 490 UV detector (Waters Associates, Inc.) operated at 254 nm and 0.1 absorbance units full scale deflection. The chromatograms were recorded and integrated by an HP Model 3390A recording integrator (Hewlett Packard, Inc.).

The HPLC system was calibrated by analyzing solutions of PAHs at five known concentrations bracketing the concentration range of interest. Calibration curves were linear with intercepts near zero ($r^2 \geq 0.97$). The HPLC calibration was checked each day prior to sample analysis. The integrated peak areas for the individual PAHs in the C18 column concentrates were entered into a Lotus 123 spreadsheet and quantified using the calibration curves

generated for the HPLC system. The truly dissolved concentration of the individual PAHs in the humic substance/PAH solutions were calculated from the concentrations measured by the HPLC procedure for the C18 column concentrates.

The K_{doc} values for the different HOCs with each humic substance were calculated using the truly dissolved HOC concentration measured by the C18 column technique in Equation 4.1. The amount of HOC associated with the humic substance was calculated based on the measured truly dissolved HOC concentration and the known amount of HOC added to each partitioning experiment.

Results and Discussion

The concentrations of the truly dissolved PCB congeners and PAHs in the humic substance/HOC solutions measured by the C18 column procedure were used in equation 4.1 to calculated the K_{doc} values for these compounds with the different sources of humic substances (Tables 4.2 and 4.3, for PCBs and PAHs, respectively). The $\log(K_{doc})$ values measured for the humic substances and HOCs in the current study are within the ranges reported for interactions of dissolved humic substances from a variety of sources with HOCs (Carter and Suffet, 1982; Kile and Chiou, 1983; Landrum *et al.*, 1984; Gaurthier *et al.*, 1986; Morehead *et al.*, 1986; Evans, 1988; Lara and Ernst, 1989; McCarthy *et al.*,

Table 4.2. Mean log(K_{doc}) values (mL water/g carbon) of polychlorinated biphenyl for selected humic substances. Standard errors are in parentheses (n=4). Octanol/water partition coeffficents (K_{ow}) and aqueous solubility (S) for each compound are included.

PCB Congener #	47	77	98	101	114	126	153	154	171	200	Mean
log(Kow) (2)	5.85	6.36	6.13	6.38	6.65	6.89	6.92	6.76	7.11	7.27	$\log(K_{doc}/K_{ow})$[1]
log(S) (3)	-0.338	-0.903	-0.648	-0.925	-1.223	-1.489	-1.522	-1.345	-1.732	-1.909	
Aldrich	3.86 (0.09)	4.49 (0.02)	4.24 (0.10)	4.75 (0.03)	4.58 (0.06)	4.90 (0.01)	5.14 (0.03)	4.78 (0.03)	4.86 (0.04)	5.09 (0.01)	0.704
IHSS Humic Acid	3.67 (0.14)	3.79 (0.02)	3.58 (0.13)	4.04 (0.01)	4.13 (0.03)	4.48 (0.01)	4.23 (0.01)	4.23 (0.16)	4.58 (0.10)	4.80 (0.09)	0.625
Pine Mt. Soil	3.06 (0.28)	3.50 (0.03)	3.28 (0.09)	3.54 (0.04)	3.62 (0.06)	3.97 (0.05)	4.00 (0.05)	3.76 (0.14)	3.95 (0.17)	4.40 (0.08)	0.558
Orange. Hts. Soil	3.05 (0.05)	3.70 (0.02)	3.45 (0.12)	3.74 (0.08)	3.84 (0.08)	4.20 (0.10)	4.32 (0.06)	4.12 (0.22)	4.32 (0.12)	4.72 (0.03)	0.593
Newnans Lake Sed. 11	3.52 (0.07)	3.67 (0.04)	3.71 (0.18)	3.82 (0.07)	3.81 (0.02)	3.79 (0.05)	4.27 (0.02)	4.02 (0.15)	4.26 (0.11)	4.38 (0.11)	0.592
Newnans Lake Sed. 16	3.72 (0.02)	4.20 (0.29)	3.89 (0.20)	3.95 (0.05)	3.96 (0.14)	4.20 (0.07)	4.19 (0.07)	4.32 (0.02)	4.41 (0.03)	4.57 (0.01)	0.625
Newnans Lake Sed. 18	3.43 (0.04)	3.57 (0.05)	3.66 (0.03)	3.75 (0.12)	3.78 (0.09)	3.92 (0.07)	4.08 (0.03)	3.77 (0.05)	4.13 (0.01)	4.24 (0.04)	0.578
Santa Fe River DOC	3.27 (0.19)	3.76 (0.02)	3.62 (0.16)	3.83 (0.03)	3.61 (0.11)	4.41 (0.03)	4.29 (0.02)	4.03 (0.27)	4.33 (0.15)	4.58 (0.14)	0.598

Table 4.2. (continued)

PCB Congener #	47	77	98	101	114	126	153	154	171	200	$\log(K_{doc}/K_{ow})$[1]
St. Marys River DOC	3.40 (0.16)	4.12 (0.03)	3.75 (0.08)	3.87 (0.03)	4.21 (0.06)	4.50 (0.05)	4.30 (0.05)	4.25 (0.16)	4.31 (0.14)	4.60 (0.07)	0.622
Orange. Hts. DOC	3.48 (0.19)	4.34 (0.03)	3.81 (0.13)	4.45 (0.02)	4.26 (0.06)	4.48 (0.03)	4.32 (0.03)	4.37 (0.19)	4.69 (0.06)	4.86 (0.05)	0.649
Newnans Lake DOC	3.38 (0.07)	3.67 (0.40)	3.45 (0.11)	3.60 (0.10)	3.68 (0.25)	3.96 (0.17)	3.93 (0.09)	3.85 (0.18)	4.07 (0.12)	4.14 (0.13)	0.569
Suwannee River FA	3.41 (0.11)	3.65 (0.01)	3.53 (0.02)	3.60 (0.01)	3.85 (0.01)	3.97 (0.02)	4.02 (0.01)	3.87 (0.07)	4.14 (0.03)	4.27 (0.06)	0.577
Suwannee River HA	3.77 (0.19)	4.08 (0.02)	3.96 (0.17)	4.12 (0.03)	4.24 (0.02)	4.38 (0.03)	4.60 (0.01)	4.21 (0.21)	4.48 (0.11)	4.59 (0.13)	0.640

[1] Mean $\log(K_{doc}/K_{ow})$ for each compound.
[2] $\log(K_{ow})$ values from Hawker and Connell, (1988).
[3] Log(Sol.) for solubility in water in units of (μmole/L) from Miller et al., (1985). All solubilities are for supercooled liquids.

Table 4.3. Mean log(K_{doc}) values (mL water/g carbon) of polynulcear aromatic hydrocarbon for selected humic substances. Standard errors are in parentheses (n=4). Octanol/water partition coeffficents (K_{ow}) and aqueous solubility (S) for each compound are included.

	Phenan-threne	Anthra-cene	Pyrene	Chrysene	Benzo-(a)-pyrene	Dibenz-(a,h)-anthracene	Benzo(k)-fluor-anthene	Mean log(K_{doc}/K_{ow})[1]
log(Kow) [2]	4.46	4.45	5.18	5.61	5.98	5.97	6.84	
log(S) [3]	1.121	1.600	1.120	0.220	-0.338	-0.351	-0.849	
Aldrich	3.60 (0.51)	3.76 (0.48)	4.17 (0.28)	4.45 (0.20)	4.91 (0.30)	5.19 (0.54)	5.34 (0.61)	0.814 (.031)
IHSS Humic Acid	3.74 (0.16)	3.61 (0.05)	3.97 (0.17)	4.28 (0.07)	4.43 (0.13)	4.70 (0.25)	5.01 (0.23)	0.758 (.019)
Pine Mt. Soil	3.60 (0.41)	3.43 (0.45)	3.80 (0.29)	4.06 (0.09)	4.30 (0.27)	4.62 (0.53)	4.55 (0.60)	0.723 (.035)
Orange. Hts. Soil	3.70 (0.27)	3.63 (0.15)	3.87 (0.30)	4.05 (0.06)	4.20 (0.29)	4.74 (0.50)	4.61 (0.52)	0.728 (.041)
Newnans Lake Sed. 11	3.56 (0.07)	3.58 (0.06)	3.80 (0.06)	4.04 (0.17)	4.08 (0.18)	4.43 (0.14)	4.67 (0.18)	0.712 (.025)
Newnans Lake Sed. 16	3.60 (0.15)	3.58 (0.17)	3.84 (0.17)	4.10 (0.28)	4.12 (0.28)	4.28 (0.08)	4.58 (0.11)	0.710 (.027)

Table 4.3. (continued)

	Phenanthrene	Anthracene	Pyrene	Chrysene	Benzo-(a)-pyrene	Dibenz-(a,h)-anthracene	Benzo(k)-fluoranthene	Mean $\log(K_{doc})/K_{ow})$[1]
Newnans Lake Sed. 18	3.55 (0.05)	3.30 (0.02)	3.79 (0.08)	4.03 (0.19)	4.03 (0.04)	4.14 (0.03)	4.55 (0.09)	0.697 (.025)
Santa Fe River DOC	3.05 (0.01)	3.14 (0.01)	3.47 (0.02)	3.92 (0.03)	3.94 (0.01)	4.06 (0.05)	4.57 (0.01)	0.675 (.013)
St. Marys River DOC	3.34 (0.01)	3.42 (0.11)	3.71 (0.03)	3.78 (0.03)	4.32 (0.01)	4.58 (0.07)	4.71 (0.07)	0.714 (.032)
Orange. Hts. DOC	3.55 (0.01)	3.87 (0.10)	3.74 (0.10)	4.08 (0.02)	4.31 (0.02)	4.55 (0.17)	4.76 (0.12)	0.726 (.021)
Newnans Lake DOC	3.93 (0.12)	4.03 (0.09)	3.85 (0.13)	4.14 (0.07)	4.35 (0.09)	4.36 (0.11)	4.58 (0.08)	0.722 (.026)
Suwannee River FA	3.84 (0.26)	3.72 (0.15)	4.04 (0.14)	4.33 (0.07)	4.22 (0.13)	4.33 (0.11)	4.73 (0.05)	0.735 (.035)
Suwannee River HA	3.74 (0.15)	3.44 (0.42)	4.00 (0.05)	4.25 (0.11)	4.26 (0.04)	4.41 (0.10)	4.79 (0.06)	0.736 (.027)

[1] Mean $\log(K_{doc}/K_{ow})$ for each compound.
[2] $\log(K_{ow})$ values from Shaul, (1991).
[3] Log(S) for solubility in water in units of (μmole/L) from Miller et al., (1985) except for Benzo(k)fluoranthene which was calculated using Equation 2-18 from Lyman et al., (1990). All solubilities are for supercooled liquids.

1989). A number investigations of the association of Suwannee River Fulvic and Humic Acids with HOCs have been reported (Chiou *et al.*, 1986; Gauthier *et al.*, 1987; Kile *et al.*, 1989; Kile and Chiou, 1989). Gauthier *et al.* (1987) reported a $\log(K_{doc})$ of 5.02 for the interaction between pyrene and Suwannee River Fulvic Acid measured using a fluorescence quenching technique. The $\log(K_{doc})$ value measured in the current study for pyrene with the Suwannee River Fulvic Acid was 4.04 (Table 4.3). The difference between the K_{doc} value for pyrene with Suwannee River Fulvic Acid reported by Gauthier *et al.* (1987) and that obtained in the current study may be due to differences in the experimental conditions. The K_{doc} values measured using fluorescence quenching techniques have generally been higher in direct comparisons with those measured using the C18 column method (Gauthier *et al.*, 1986; Backhus and Gschwend, 1990). In addition, the Suwannee River Fulvic Acids may be different sources of humic material based on the reported differences in elemental analyses. Moreover, Gauthier *et al.* (1987) did not specify the pH and ionic strength at which the K_{doc} was measured. The K_{doc} values of HOCs with humic substances have been reported to decrease with increasing pH (Carter and Suffet, 1982; Kile and Chiou, 1989; Jota and Hassett, 1991) and decrease as ionic strength is decreased (Carter and Suffet, 1982; Jota and Hassett, 1991). The $\log(K_{doc})$ for PCB 52 with Aldrich humic acid has

been reported to decrease by 50% as the pH was increased from 6 to 9 and to increase by 30% as the ionic strength was increased from 0.01 to 0.5 M NaCl (Jota and Hassett, 1991).

The effect of pH on K_{doc} values can be illustrated by comparing the results from the current investigation with those of Kile *et al.* (1989) for Suwannee River Fulvic and Humic Acids. These authors indicated that these humic substances were obtained from the IHSS. Therefore, it can be assumed that these humic substances are the same sources as those used in the current investigation. Kile *et al.* (1989) reported $\log(K_{doc})$ values for PCB congener 101 of 4.10 and 4.07, for Suwannee River Fulvic and Humic Acids respectively. These values were obtained from experiments performed at pH 5.7 for the fulvic acid, pH 5.5 for the humic acid and an unspecified ionic strength. The $\log(K_{doc})$ values measured in the current study for PCB 101 were 3.60 with Suwannee River Fulvic Acid and 4.12 with Suwannee River Humic Acid (Table 4.2). The $\log(K_{doc})$ decreased for PCB 101 with Suwannee River Fulvic Acid (-0.50) with a pH increase of 1.3 units while the $\log(K_{doc})$ for Suwannee River Humic Acid remained essentially unchanged.

The decrease in $\log(K_{doc})$ for PCB 101 with Suwannee River Fulvic acid with changing pH is consistent with the reported larger decrease in $\log(K_{doc})$ for DDT with fulvic acids than with humic acids as the pH was increased. Kile and Chiou (1989) observed a decrease in $\log(K_{doc})$ for DDT

from 4.39 to 3.88 for an aquatic fulvic acid as the pH was increased from 6.5 to 8.5, while over the same pH increase the $\log(K_{doc})$ for DDT with a soil humic acid only decreased from 4.87 to 4.79.

The effect of pH on the association of HOCs with humic substances is consistent with the known effects of pH on the behavior of aquatic humic substances (Carter and Suffet, 1982; Kile *et al.*, 1989; Jota and Hassett, 1991). An increase in pH causes an increase in the number of ionized acidic functional groups present for a particular humic substance. Humic substances become more hydrophilic as the acidic functional groups become ionized (Jota and Hassett, 1991). The macromolecular configurations of humic substances in solution have been reported to change from "spherocolloids" to flexible linear molecules with increasing pH or decreasing ionic strength (Ghosh and Schnitzer, 1980). The linear configuration is due to the increased ionization of acidic functional groups. The association of HOCs would be expected to decrease as the humic substance itself becomes more hydrophilic.

The decrease in $\log(K_{doc})$ for PCB 101 with Suwannee River Fulvic Acid as the pH increases while the $\log(K_{doc})$ with Suwannee River Humic Acid remains constant is also consistent with the earlier compositional data obtained for these two humic substances. The higher carboxylic acid content (Tables 3.8, 3.9 and 3.10) and total acidity (Table

3.3) of Suwannee River Fulvic Acid compared to Suwannee River Humic Acid would cause a more linear configuration for the former as the pH was increased. The decease in K_{doc} for Suwannee River Fulvic Acid with PCB 101 likely results from the decrease in the hydrophobic character of this humic substance as the pH increases.

Influence of HOC Hydrophobic Character on K_{doc}

A number of researchers have demonstrated that, for a particular humic substance, the K_{doc} for different solutes is related to the hydrophobicity of the solute. Relationships have been reported that indicate, for a particular humic substance, K_{doc} increases as the solute solubility decreases (Chiou *et al.*, 1986; Morehead *et al.*, 1986; Kile *et al.*, 1989; Lara and Ernst, 1989; Lee and Farmer, 1989), K_{ow} increases (Landrum *et al.*, 1987; Evans, 1988; Lara and Ernst, 1989) and total surface area increases (Lara and Ernst, 1989). These relationships are similar to those reported for the sorption of HOCs by soil and sediment organic matter. Numerous relationships have been reported between the carbon normalized soil partition coefficient (K_{oc}) and both the aqueous solubility (Chiou *et al.*, 1979; Chiou *et al.*, 1983) and K_{ow} (Karichkoff *et al.*, 1979; Means *et al.*, 1980; Karichkoff, 1981; Chiou *et al.*, 1983) of HOCs.

Thermodynamic Basis for Partition Coefficients

The partitioning of HOCs between water and an organic phase (e.g., soil/sediment organic matter, lipid of biota, dissolved humic substances, and immiscible organic solvents) is dependent on the free energy change on transfer between two phases (Hawker and Connell, 1988). At equilibrium, the chemical potentials of the solute (e.g., HOC) in both phases are equal (using Raoult's law conventions for activity coefficients):

$$X_o\gamma_o = X_w\gamma_w \qquad (4.2)$$

where X_o and X_w are the mole fraction of the solute in the organic and aqueous phases, respectively and γ_o and γ_w are the activity coefficients of the solute in the organic and aqueous phases, respectively. The partition coefficient is often defined as the ratio of the solute concentration in the organic phase (C_o, mole/L) to its concentration in the aqueous phase (C_w):

$$K = C_o/C_w \qquad (4.3)$$

At low solute concentration (dilute solution), the approximation can be made that X_i is equal to C_i times the molar volume (V_i) (Moore, 1972). Equation 4.2 then becomes:

$$C_o V_o \gamma_o = C_w V_w \gamma_w \qquad (4.4)$$

Solving Equation 4.4 for the concentration ratios yields an activity based partition coefficient expression:

$$K = V_w \gamma_w / V_o \gamma_o \qquad (4.5)$$

The solubility (S) of a solute in water (C_w) is $1/(\gamma_w V_w)$, substituting into Equation 4.4 yields:

$$K = 1/(S V_o \gamma_o) \qquad (4.6)$$

or

$$\log K = -\log S - \log V_o - \log \gamma_o \qquad (4.7)$$

The above discussion assumes that the two phases are pure and immiscible. However, if the organic solvent is significantly soluble in the aqueous phase the equation becomes (Chiou *et al.*, 1982):

$$\log K = -\log S - \log V_o - \log \gamma_o + \log(\gamma_w^*/\gamma_w) \qquad (4.8)$$

where γ_w^* denotes the solute activity coefficient in the solvent saturated aqueous phase. However, if the HOC forms an ideal solution in the organic phase and the HOC

solubility is the same in water and the organic saturated aqueous phase, then the last two terms in Equation 4.8 become negligible (Chiou *et al.*, 1982). The plot of logK vs. logS then represents an ideal solution and would have a slope of -1 and an intercept of -log V_o.

Linear relationships have been reported between logS and log(K_{ow}) for a large number of different classes of HOCs (Lyman *et al.*, 1990). However, the slopes of the reported lines were significantly lower than -1 (Chiou *et al.*, 1977; Banerjee *et al.*, 1980). Also, different classes of HOCs gave non-parallel slopes (Chiou *et al.*, 1982). These results are indicative of non-ideal behavior. The relative importance of the different terms in equation 4.8 to the relationship between K and S in the octanol/water system have been extensively studied (Chiou *et al.*, 1977; Mackay, 1977; Banerjee *et al.*, 1980; Chiou et al, 1982; Miller et al, 1984; Miller *et al.*, 1985).

Mackay (1977) was able to explain a major portion of the apparent deviation from ideal behavior in these relationships by using the super-cooled liquid solubilities for solid solutes. The effect of melting point on solubility always causes a downward deviation of the plotted solubility without affecting the partition coefficient, since the melting point affects both solvents equally (Mackay, 1977; Chiou *et al.*, 1982). Using super-cooled liquid solubilities, Chiou *et al.* (1982) reported a linear

relationship between log(S) and log(K_{ow}) for a diverse group of HOC (n= 36), including both PCBs and PAHs:

$$\log(K_{ow}) = -0.862\log(S) + 5.29 \qquad (4.9)$$

for solubility expressed in μmole/L ($r^2 = 0.99$). The slope was significantly different from -1 ($p < 0.0001$). Similar results have been reported for a series of chlorobenzenes and PCBs (slope = -0.795, $r^2 = 0.96$, n = 28) (Miller *et al.*, 1984).

The downward deviation of these relationships from the ideal line has been attributed the $-\log\gamma_o$ term in Equation 4.8 (Chiou *et al.*, 1982; Miller *et al.*, 1985). The γ_w^*/γ_w term for the octanol/water system has been shown to be constant and equal to one for a large number of different HOC (Banerjee *et al.*, 1980; Chiou *et al.*, 1982; Miller *et al.*, 1985). Therefore, this term drops out of Equation 4.8 to yield Equation 4.7 and the deviation from ideal behavior in the octanol/water system can be considered to be due to the variation in the γ_o for HOCs (Miller *et al.*, 1984).

In summary, the factors which are important in predicting the partitioning of an HOC between octanol and water, in decreasing order of importance, are HOC water solubility, compatibility of the HOC with water-saturated octanol (γ_o) and the effect of octanol dissolved in water on the water solubility (γ_w^*/γ_w). This last term has been shown

to be negligible compared with the other two (Banerjee *et al.*, 1980; Chiou *et al.*, 1982; Miller *et al.*, 1985).

Similarity between octanol and dissolved humic substances as solvents in HOC partitioning

The log(S) correlation with $\log(K_{ow})$ reported by Chiou *et al.* (1982) (Equation 4.8) was based on the pooled data for 36 different HOCs, including several homologous series, all of which were substituted benzenes. However, different homologous series have been reported to exhibit linear relationships between log(S) and $\log(K_{ow})$ with parallel slopes but different intercepts (Chiou *et al.*, 1982; Miller *et al.*, 1985). The results of the regression of log(S) with $\log(K_{ow})$ for the PCBs and PAHs used in this study (Table 4.4 and Figure 4.1) confirm that these two homologous series of HOCs have essentially the same slopes but significantly different intercepts.

The difference between the intercepts for these relationships can be attributed to the $-\log\gamma_o$ $-\log V_o$ terms in Equation 4.7 (Miller *et al.*, 1984). Since the molar volume of octanol (V_o) remains constant, the intercepts of these two lines can be used to solve for the ratio of the activities of the PCBs and PAHs in octanol. This can be done by setting the intercept terms in Equation 4.7 equal to the intercept values obtained from the regression analyses (Table 4.4) for octanol and solving for the ratio of activity coefficients for PCBs and PAHs:

Table 4.4. Regression summaries of solubilities (log(S); μmole/L) against HOC partition coefficients (log(K_{doc}); mL/g) for polychlorinated biphenyl congeners (n = 10) and polynuclear aromatic hydrocarbons (n = 7). See Tables 4.2 and 4.3. Standard errors are in parentheses. All regressions are significant ($p < 0.001$).

	Polychlorinated Biphenyl Congeners			Polynuclear Aromatic Hydrocarbons		
	Slope	Intercept	r^2	Slope	Intercept	r^2
Aldrich	-0.726 (0.114)	3.800 (0.147)	0.840	-0.704 (0.100)	4.742 (0.093)	0.910
IHSS Humic Acid	-0.761 (0.088)	3.237 (0.113)	0.900	-0.535 (0.057)	4.441 (0.053)	0.950
Pine Mt. Soil	-0.758 (0.068)	2.803 (0.087)	0.940	-0.481 (0.059)	4.225 (0.055)	0.930
Orange Hts. Soil	-0.971 (0.058)	2.776 (0.074)	0.970	-0.425 (0.084)	4.268 (0.078)	0.840
Newnans Lake Sed. 11	-0.536 (0.095)	3.291 (0.123)	0.790	-0.423 (0.069)	4.175 (0.065)	0.880
Newnans Lake Sed. 16	-0.474 (0.082)	3.570 (0.106)	0.810	-0.376 (0.051)	4.150 (0.048)	0.920
Newnans Lake Sed. 18	-0.490 (0.061)	3.242 (0.079)	0.890	-0.418 (0.064)	4.063 (0.059)	0.900
Santa Fe River DOC	-0.794 (0.110)	3.017 (0.142)	0.870	-0.555 (0.083)	3.936 (0.078)	0.900

Table 4.4. (continued)

	Polychlorinated Biphenyl Congeners			Polynuclear Aromatic Hydrocarbons		
	Slope	Intercept	r^2	Slope	Intercept	r^2
St. Marys River DOC	-0.693 (0.086)	3.300 (0.111)	0.890	-0.562 (0.091)	4.183 (0.084)	0.890
Orange Hts. DOC	-0.722 (0.128)	3.436 (0.166)	0.800	-0.433 (0.087)	4.279 (0.081)	0.840
Newnans Lake DOC	-0.513 (0.035)	3.155 (0.045)	0.970	-0.337 (0.044)	4.253 (0.045)	0.980
Suwannee River FA	-0.557 (0.029)	3.160 (0.370)	0.980	-0.340 (0.062)	4.295 (0.058)	0.860
Suwannee River HA	-0.522 (0.066)	3.606 (0.860)	0.890	-0.456 (0.067)	4.291 (0.063)	0.900
Octanol	-0.904 (0.020)	5.544 (0.010)	0.990	-0.898 (0.121)	5.855 (0.175)	0.920

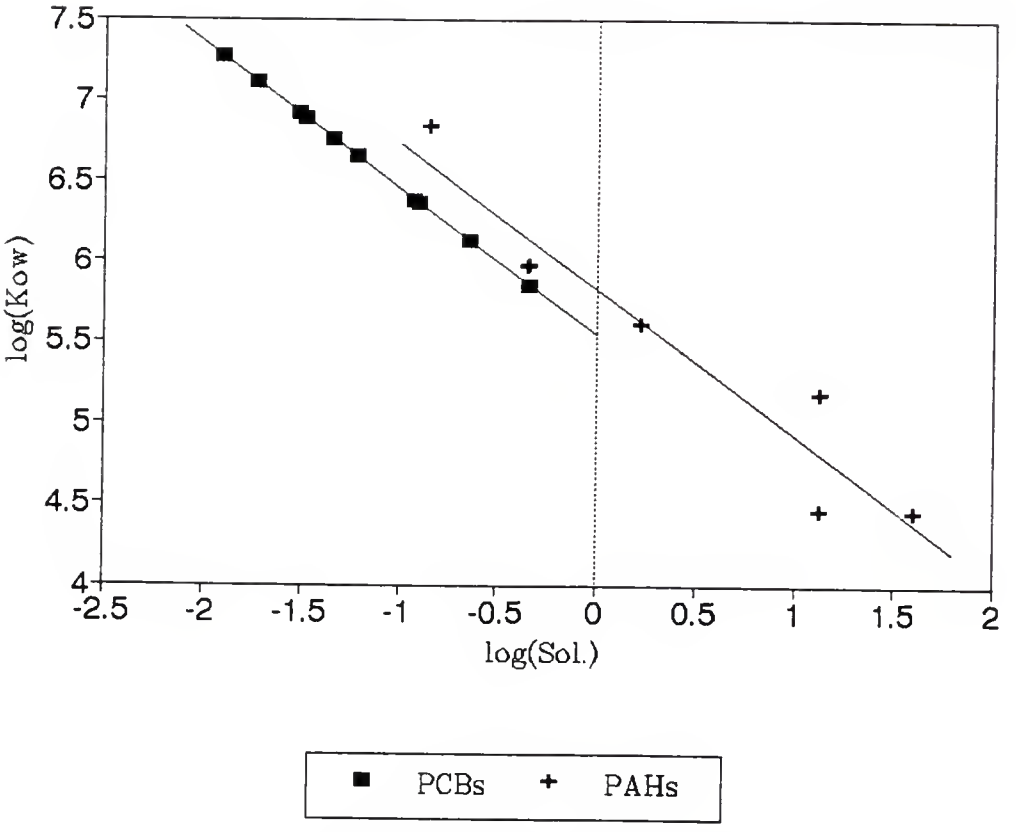

Figure 4.1. The log(S) was correlated with the $\log(K_{ow})$ for the PCBs (r^2 = 0.99, intercept = 5.544, slope = -0.904, $p < 0.001$) and PAHs (r^2 = 0.92, intercept = 5.855, slope = -0.898, $p < 0.001$). The slopes of the lines are essentially equal. However, the intercepts are significantly different.

$$-\log\gamma_{o,PCB} - \log V_o = 5.544 \qquad (4.10)$$

$$-\log\gamma_{o,PAH} - \log V_o = 5.855 \qquad (4.11)$$

subtract Equation 4.11 from Equation 4.10

$$-\log\gamma_{o,PCB} + \log\gamma_{o,PAH} = -0.311 \qquad (4.12)$$

$$\log(\gamma_{o,PCB}/\gamma_{o,PAH}) = 0.311 \qquad (4.13)$$

The results of this procedure indicate that the activity coefficients of the PCBs are 2 times higher in octanol than the activity coefficients of PAHs.

Partitioning description of dissolved humic substance/HOC interactions

If one considers the dissolved humic substances as an organic phase into which partitioning can occur from water, then the application of Equation 4.7 could be used to investigate the relationship between log(S) and log(K_{doc}) for the humic substances in the current study. Equation 4.7 would be expressed as:

$$\log(K_{doc}) = -\log(S) - \log\gamma_{doc} - \log V_{doc} \qquad (4.14)$$

The term for γ_w^*/γ_w was not included in Equation 4.14 based on the findings that it is unity for the octanol/water system discussed above (Banerjee *et al.*, 1980; Chiou *et al.*, 1982; Miller *et al.*, 1985) and other solvents (Chiou, 1985). Also, this term would only be expected to be important when the organic solvent dissolves at several percent (by weight) in the aqueous phase (Miller *et al.*, 1985). The concentrations of the humic substances used in this study were on the order of 0.005% (by weight). Therefore the γ_w^*/γ_w term is negligible in these interactions.

The linear relationships between log(S) and log(K_{ow}) for Suwannee River Humic acid with both the PCBs and PAHs (Figure 4.2) are typical for the humic substances investigated in this dissertation research (Figures A2.1 to A2.12). The results of correlations between log(S) and log(K_{doc}) for the PCBs and PAHs with the humic substances in this dissertation research (Table 4.4) indicate that the humic substances behave as solvents which are similar to octanol in that, for all cases, the intercept of the regression lines for a particular humic substance is larger for the PAHs than for the PCBs. Therefore, based on calculations analogous to those performed in Equations 4.10 through 4.13, these results indicate that the PCBs have higher activities for partitioning into a particular humic substance than do the PAHs. This finding is significant since the humic substances come from a wide variety of

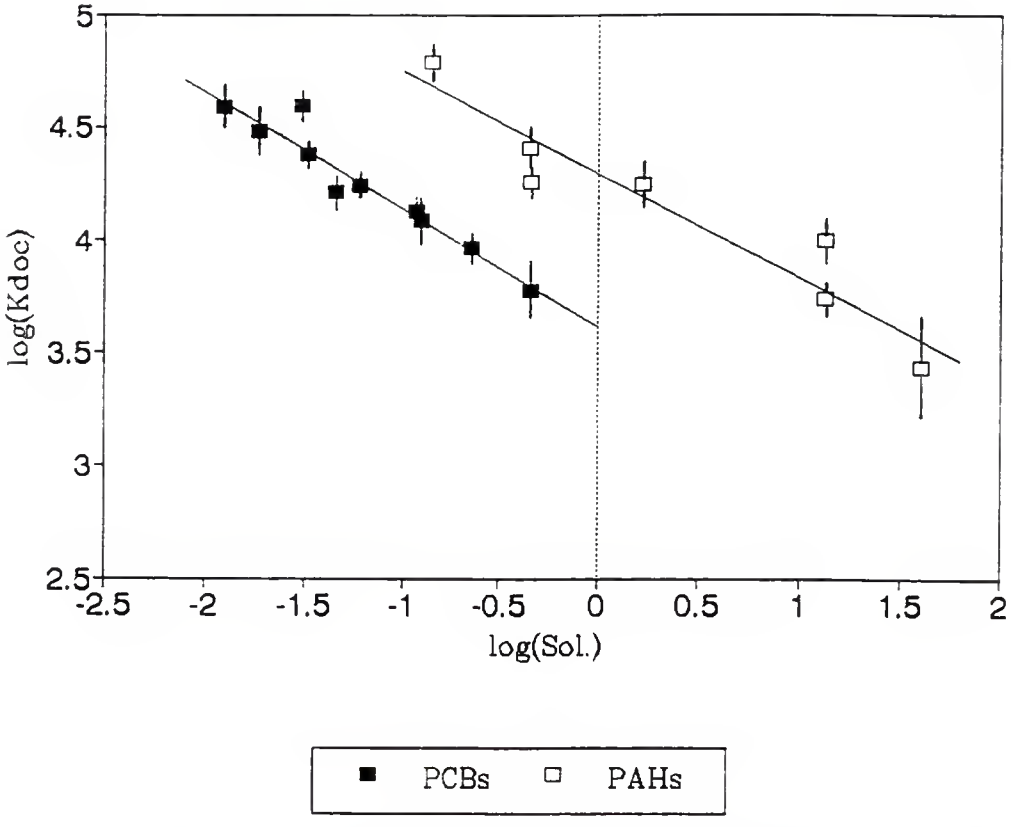

Figure 4.2. The log(S) was correlated with the $\log(K_{doc})$ for the Suwannee River Humic Acid and both the PCBs (r^2 = 0.93, slope = -0.525, intercept = 3.611, $p < 0.01$) and PAHs (r^2 = 0.90, slope = -0.456, intercept = 4.291, $p < 0.01$). The slopes are not significantly different, statistically, while the intercepts are significantly different (see Table 4.4). Similar plots were obtained for the other humic substances investigated (see Appendix B, Figures B2.1 to B2.12).

sources and are compositionally and structurally varied. The similarity between the behavior of octanol and that of these dissolved humic substances, relative to their interactions with HOCs, strongly supports a partition mechanism for these interactions.

Variation in Partitioning of HOCs with Different Dissolved Humic Substances

As discussed above, linear correlations have been reported between $\log(K_{ow})$ and $\log(K_{oc})$ for different soils and sediments (Karichkoff *et al.*, 1979; Means *et al.*, 1980; Karichkoff, 1981; Chiou *et al.*, 1983) and $\log(K_{doc})$ for individual dissolved humic substances (Landrum *et al.*, 1987; Evans, 1988; Lara and Ernst, 1989). This is not surprising since the low level of water solubility of HOCs is the major determinant in both Kow and Koc (Karichkoff, 1981; Chiou *et al.*, 1983) as well as in K_{doc} for individual humic substances (Table 4.4 and Landrum *et al.*, 1987; Evans, 1988). However, there appear to be other factors that influence the partitioning of HOCs between soil/sediment and dissolved humic substances that are quite different (Chiou *et al.*, 1986; Kile and Chiou, 1989).

A major factor in partitioning of HOCs between water and soil is the amount of organic carbon present (Means *et al.*, 1980; Chiou *et al.*, 1983). Karickhoff (1981) reported a strong linear correlation ($r^2 = 0.997$) between $\log(K_{ow})$ and

$\log(K_{oc})$ for substituted benzenes and PAHs with a wide variety of soils and sediments (n =17):

$$\log(K_{oc}) = 0.989\log(K_{ow}) - 0.346 \qquad (4.15)$$

A number of correlations of this type with similar slopes and intercepts have been reported for soils and sediments (Lyman *et al.*, 1990). Karickhoff (1981) found that Equation 4.15 predicted experimental K_{oc} values within a factor of 2. These strong correlations indicate that the different soil and sediment organic matter investigated have similar activity coefficients for HOCs.

Linear correlations have also been reported between the $\log(K_{ow})$ and $\log(K_{doc})$ for HOCs with a single source of dissolved humic substances. Lara and Earnst (1989) reported a strong correlation (r^2 = 0.95) between $\log(K_{ow})$ and $\log(K_{doc})$ for a series of PCBs (n =14) and an isolated marine humic substance. Chiou *et al.* (1986) reported linear correlations for four different sources of dissolved humic substances between these two partition coefficients for a series of substituted benzenes. However, these authors reported that there was a significant variation in $\log(K_{doc})$ for a particular HOC with different sources. Other researchers have also reported wide variations in $\log(K_{doc})$ values measured for a single HOC with different sources of

HOC (Chiou *et al.*, 1987; Gauthier *et al.*, 1987; Landrum *et al.*, 1987; Evans, 1988).

The results of correlations between $\log(K_{ow})$ and $\log(K_{doc})$ values measured for HOCs with different sources of dissolved humic substances indicate that the organic carbon content of the humic substances, although important, is not the major controlling factor in these interactions (Whitehouse, 1985; Chiou *et al.*, 1986; Malcolm and MacCarthy, 1986; Chiou *et al.*, 1987; Gauthier *et al.*, 1987; Alberts *et al.*, 1989; McCarthy *et al.*, 1989). The variation in $\log(K_{doc})$ values reported for a given HOC with different sources of dissolved humic substances have been reported to be greater than an order of magnitude (Chiou *et al.*, 1987; Gauthier *et al.*, 1987; Landrum *et al.*, 1987; Evans, 1988). These observations have prompted a number of investigators to postulate that the variations in K_{doc} for a given HOC with different sources of dissolved humic substances is due to variation in the humic substances structure and composition (Whitehouse, 1985; Chiou *et al.*, 1986; Malcolm and MacCarthy, 1986; Chiou *et al.*, 1987; Gauthier *et al.*, 1987; Alberts *et al.*, 1989).

Only a portion of the variation in K_{doc} values reported for HOCs with different humic substances has been attributed to the hydrophobicity of the HOCs. Landrum *et al.* (1987) found a correlation ($r^2 = 0.49$, $p < 0.01$, $n = 22$) between the $\log(K_{ow})$ and $\log(K_{doc})$ for four different HOCs with four

different humic substances obtained from interstitial waters of Lake Michigan sediments. These authors indicated that K_{doc} for a particular HOC with different humic substances varied over an order of magnitude. Evans (1988) reported a relationship between $\log(K_{ow})$ and $\log(K_{doc})$ for K_{doc} values measured with 12 freshwater water humic sources and a number of PCB congeners. Again the relationship was not strong ($r^2 = 0.49$, $p < 0.01$, $n = 39$) and the measured K_{doc} values for a given PCB congener varied over an order of magnitude with different sources of humic substances.

Results of the correlation between $\log(K_{ow})$ and $\log(K_{doc})$ obtained in the current study were similar to those of Landrum *et al.* (1987) and Evans (1988). The $\log(K_{doc})$ values for the different humic sources were pooled and regressed against the $\log(K_{ow})$ values of the HOCs (Figure 4.3). The relationship between the $\log(K_{ow})$ and $\log(K_{doc})$ for the ten PCB congeners with the thirteen humic substances ($r^2 = 0.54$, $p < 0.01$, $n = 130$) was weaker than the relationship observed for the seven PAHs with the same humic substances ($r^2 = 0.72$, $p < 0.01$, $n = 91$).

The observed variation of $\log(K_{doc})$ for a particular HOC with the different humic substances in the current study was approximately one order of magnitude (Figure 4.3; Tables 4.2 and 4.3). The relationships between $\log(K_{ow})$ and $\log(K_{doc})$ obtained in the current study, in combination with the findings previously reported (Landrum *et al.*, 1987; Evans,

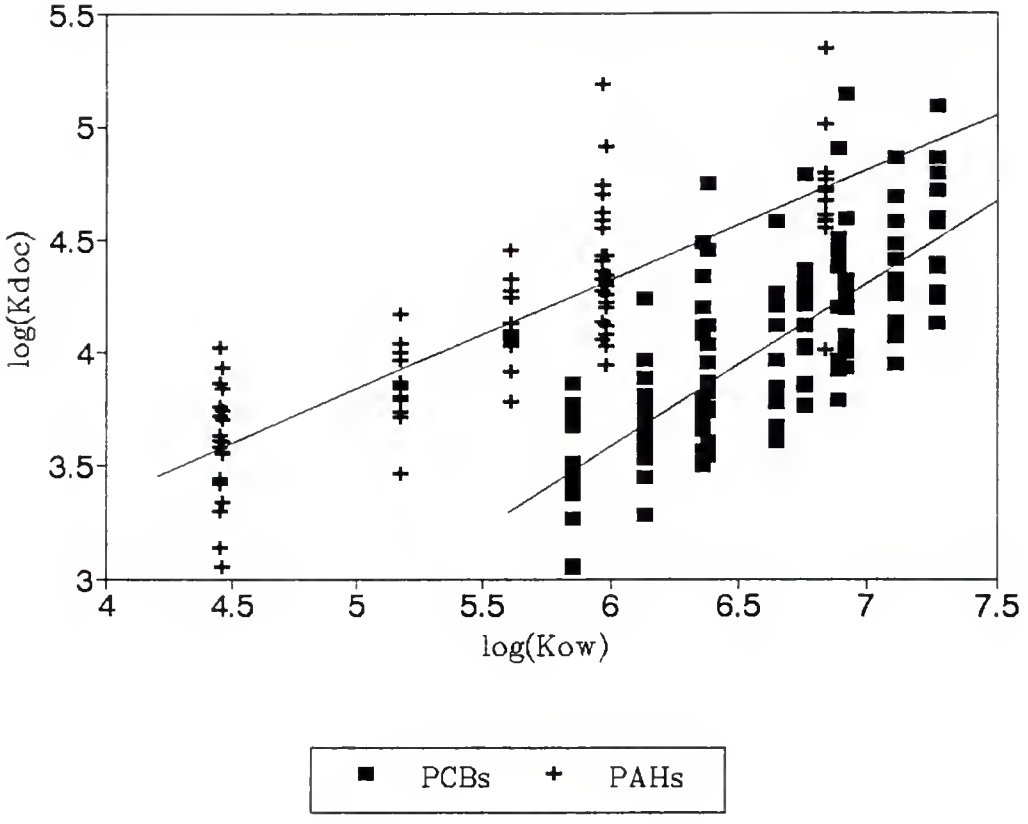

Figure 4.3. The log(K_{ow}) was correlated with the log(K_{doc}) for the pooled data from all humic substances investigated. The was a significant difference between the slopes of the lines for the PCBs (r^2 = 0.54, slope = 0.724, intercept = -0.757, $p < 0.01$) and PAHs (r^2 = 0.72, slope = 0.483, intercept = 1.427, $p < 0.01$). These results are similar to those reported by Landrum et al. (1987).

1988) indicate that from 50 to 70% of the variation in $\log(K_{doc})$ values measured for a particular HOC with different humic substances can be attributed to the hydrophobic character of the HOC (as expressed by $\log(K_{ow})$). Further, the differences in the intercepts of the correlations between $\log(S)$ and $\log(K_{doc})$ for the PCBs and PAHs (Table 4.4) with different humic substances indicate that the PCBs have higher activities than the PAHs for interactions with each humic substance investigated.

The strong linear relationships between the hydrophobic character of HOCs (solubility and K_{ow}) and the organic carbon normalized partition coefficients measured for soils/sediments (K_{oc}) with different sorbent sources indicate that the organic carbon content of the soil/sediment organic matter and the hydrophobic character of the HOCs are sufficient to predict their interactions (Karichkoff *et al.*, 1979; Means *et al.*, 1980; Karichkoff, 1981). However, the linear relationships between K_{ow} and the carbon normalized partition coefficients (K_{doc}) measured for HOCs with different sources of dissolved humic substances are weaker and variable (Figure 4.3 and Landrum *et al.*, 1987; Evans, 1986). The large variations in the K_{doc} values for a particular HOC with different humic substances observed in this study and reported in the literature indicate that factors other than the hydrophobicity of the HOCs and the carbon content of the humic substances must be

considered to explain these interactions. These other factors and their effects on the interactions between HOCs and dissolved humic substances must be identified before a predictive model for K_{doc} can be developed.

Influence of the Structure and Composition of Humic Substances on Interactions with HOCs

The interactions between dissolved organic carbon and HOCs in aqueous systems has been a subject of a large number of investigations in recent years. As discussed above, these interactions influence the ultimate fate of HOCs in the environment. However, efforts to predict the interactions of HOCs with different sources of DOC have been unsuccessful. Attempts to extend models of HOC interactions with soils, based on the HOC hydrophobicity (K_{ow}) and the organic carbon content of the organic matter, to DOC have failed due to the large variation in K_{doc} observed for a particular HOC with different DOC sources.

Considerable variation in K_{doc} values for a number of HOCs has been reported for their interactions with a large number of DOC sources (Carter and Suffet, 1982; Whitehouse, 1985; Chiou *et al.*, 1986; Gauithier *et al.*, 1987; Evans, 1988; Kile and Chiou, 1989; McCarthy *et al.*, 1989). Landrum *et al.* (1987) reported that 49% of the variation in K_{doc} for four different HOCs with four sources of DOC could be accounted for by the K_{ow} of the HOCs. This result is similar to the variation observed for the K_{doc} values

obtained for both the PCBs and PAHs in the current study with the thirteen different humic substances. The K_{ow} of the HOCs accounted for 54% of the variation in K_{doc} for the PCBs and 72% for the PAHs (Figure 4.3). The remaining variation in these interactions has been postulated to be due to the structure and composition of the DOC (Whitehouse, 1985; Chiou *et al.*, 1986; Gaurthier *et al.*; 1987; Kile and Chiou, 1989; McCarthy *et al.*, 1989). Although many researchers have come to this conclusion, few have attempted to experimentally investigate the effects of DOC structure and composition on their interactions with HOCs.

To investigate the relationships between humic substance structure and composition and their ability to sorb HOCs, multiple correlation analyses were undertaken structural and compositional parameters of the humic substances measured in Chapter 3 and the $\log(K_{doc})/\log(K_{ow})$ for the HOCs. The correlations were made with the ratio of the $\log(K_{doc})/\log(K_{ow})$ to remove variation in the data due to differences in hydrophobicity within the PCB and PAH homologous series. This method of removing the variation in hydrophobicity of the HOCs from these analyses is valid based on the results of the strong linear correlations between $\log(K_{doc})$ and log(Sol) (Table 4.4) for the PCBs and PAHs with each humic sources.

Although the correlations between the HOC binding with the structural and compositional characteristics of the

humic substances are not strong (Table 4.5) they indicate that variations in these characteristics indeed affect the HOC/DOC interactions. The fact that correlations were observed for both the atomic H/C ratio and the absorptivity at 272 nm with the strength of HOC sorption was not surprising since a correlation was observed earlier between these two characteristics (Figure 3.1). Also, both aromatic and carboxylic acid functional groups influence the atomic H/C ratio and the absorptivity at 272nm (Gauthier *et al.*, 1987; Traina *et al.*, 1990). Based on the correlation coefficents for these two parameters with the K_{ow} normalized K_{doc} values, the aromatic and carboxylic acid functional group content account for between 40 and 60% of the variation in the K_{doc} values measured for the humic substances in this study with both the PCBs and PAHs.

Correlations observed for $\log(K_{doc}/K_{ow})$ with the atomic H/C ratio and the absorptivity at 272 nm are similar to results reported in the literature. McCarthy *et al.* (1989) reported a correlation ($r^2 = 0.90$) between the absorptivity at 270 nm and $\log(K_{doc})$ for benzo(a)pyrene with twelve surface and ground water dissolved humic substances. Gauthier *et al.* (1987) reported correlations between K_{doc} for pyrene and both the atomic H/C ratio ($r^2 = 0.48$) and the absorptivity at 272 nm ($r^2 = 0.90$) for fifteen different humic substances from soil, sediment and aquatic sources. The correlations observed between the sorption of HOCs and

Table 4.5. Statistical relationships between $\log(K_{doc}/K_{ow})$, and strutural and compositional characteristics of different humic substances, based on linear regression (1). All regressions significant at $p < 0.05$, except when N.S. is noted.

Independent Variable	Slope (±S.E.)	Intercept (±S.E.)	r^2
Atomic H/C Ratio (2)			
PCBs	-0.15 (0.06)	0.76 (0.06)	0.39
PAHs	-0.10 (0.06)	0.83 (0.06)	0.48
Absorptivity at 272 nm (3)			
PCBs	$2.1 \cdot 10^{-3}$ ($5.0 \cdot 10^{-4}$)	0.52 (0.03)	0.59
PAHs	$1.5 \cdot 10^{-3}$ ($5.0 \cdot 10^{-4}$)	0.66 (0.02)	0.50
IR Peak Height Ratio at 1600 cm^{-1}, pH 11 (4)			
PCBs	0.15 (0.05)	0.42 (0.06)	0.49
PAHs	0.08 (0.05)	0.63 (0.06)	N.S.
^{13}C Percent Aromatic Carbon (100-160ppm) (5)			
PCBs	$5.5 \cdot 10^{-5}$ ($1.0 \cdot 10^{-3}$)	0.61 (0.04)	N.S.
PAHs	$1.5 \cdot 10^{-3}$ ($6.0 \cdot 10^{-4}$)	0.68 (0.02)	0.52

(1) $\log(K_{doc}/K_{ow})$ values from Tables 4.2 and 4.3 for PCBs and PAHs, respectively
(2) Atomic H/C Ratio from Table 3.1
(3) Absorptivity at 272nm from Table 3.2
(4) IR Peak Height Ratio from Table 3.9
(5) ^{13}C Percent Aromatic Carbon from Table 3.10

the absorptivity at 272 and 270 nm indicate that oxygen containing functional groups, as well as aromatic carbon content, are important in these interactions.

The strongest correlation between the structure of the humic substances and the K_{doc} for pyrene reported by Gauthier *et al.* (1987) was with the aromatic carbon content measured by ^{13}C NMR. The correlation ($r^2 = 0.94$) was based on only four humic substances, three soil and one marine sediment sources. The carboxylic acid content of these four humic substances was also correlated ($r^2 = 0.85$) with the K_{doc} for pyrene. These two relationships further confirm that both aromatic carbon and carboxylic acid content are important in determining K_{doc}.

The strong correlation observed by Gauthier *et al.* (1987) between the aromatic carbon content and the K_{doc} for pyrene indicates that this structural component is important in these interactions. However, a larger data base is needed to evaluate the generality of this relationship. Data for the current study indicate a significant positive correlation ($r^2 = 0.52$) of $\log(K_{doc}/K_{ow})$ for the PAHs with the aromatic carbon content (Table 3.10, measured by ^{13}C NMR) for eight of the humic substances investigated (Table 4.5). Although this correlation is not as strong as the correlation reported by Gauthier *et al.* (1987), it does indicate that the aromatic carbon content is an important structural parameter in explaining the variation in PAH

sorption with these dissolved humic substances. However, there was no correlation (r^2 = 0.04) observed between the aromatic carbon content of these humic substances and the $\log(K_{doc}/K_{ow})$ for the PCBs investigated in this study.

The lack of correlation between the aromatic carbon content of the humic substances and the sorption of PCBs in the current study may indicate that PCB sorption is less sensitive to the aromatic carbon content of humic substances than is PAH sorption. This hypothesis is supported by the correlations for these humic substances between the IR peak height ratio at 1600 cm^{-1} at pH 11 (Table 3.9) and the $\log(K_{doc}/K_{ow})$ (Table 4.5). It should be recalled that at pH 11 this region of the IR spectrum for humic substances represents the combined absorption of carboxylate anion and aromatic carbon (Table 3.7). The correlation between these two parameters for the PCBs (r^2 = 0.49) is stronger than it is for the PAHs (r^2 = 0.22). These correlations indicate that sorption of PCBs appears to be more sensitive to the carboxylic acid content and less sensitive to the aromatic carbon content of the humic substances. These results are similar to those of McCarthy *et al.* (1989) who reported that PAHs have a greater affinity for more aromatic humic substances, while PCBs have a greater affinity for those with lower aromatic content. The correlations observed in the current research (Table 4.5) indicate that the oxygen containing functional groups of these humic substances are

important characteristics which influence their interactions with HOCs.

The relationships between humic substance structure and composition and their interactions with the PCB and PAH homologous series indicate that the observed differences between the activity coefficients of the PCBS and PAHs (Table 4.4) with these humic substances may be related to humic substance structure and composition. The relationship between $\log(\gamma_{doc,PCB}/\gamma_{doc,PAH})$ and a number of structural and compositional characteristics were investigated using correlation analysis. Two structural/compositional characteristics of the humic substances investigated were found to be strongly correlated with the $\log(\gamma_{doc,PCB}/\gamma_{doc,PAH})$, when two extreme outliers were dropped from the analyses (Orange Heights Soil and Suwannee River Humic Acid). The $\log(\gamma_{doc,PCB}/\gamma_{doc,PAH})$ was inversely correlated with both the atomic O/C ratio (Figure 4.4; $r^2 = 0.81$, $p < 0.001$) and the Total Acidity (Figure 4.5; $r^2 = 0.80$, $p < 0.001$).

The variation in the activity coefficient ratio for the PCBs and PAHs is strongly influenced by the carboxylic acid content of the humic acids investigated, based on the two correlations discussed above. These results are consistent with the reported inverse relationship between K_{doc} and the atomic O/C ratio for the interactions of two PCBs and DDT with four different humic substances (Chiou *et al.*, 1986; Chiou *et al.*, 1987). The negative slopes of these

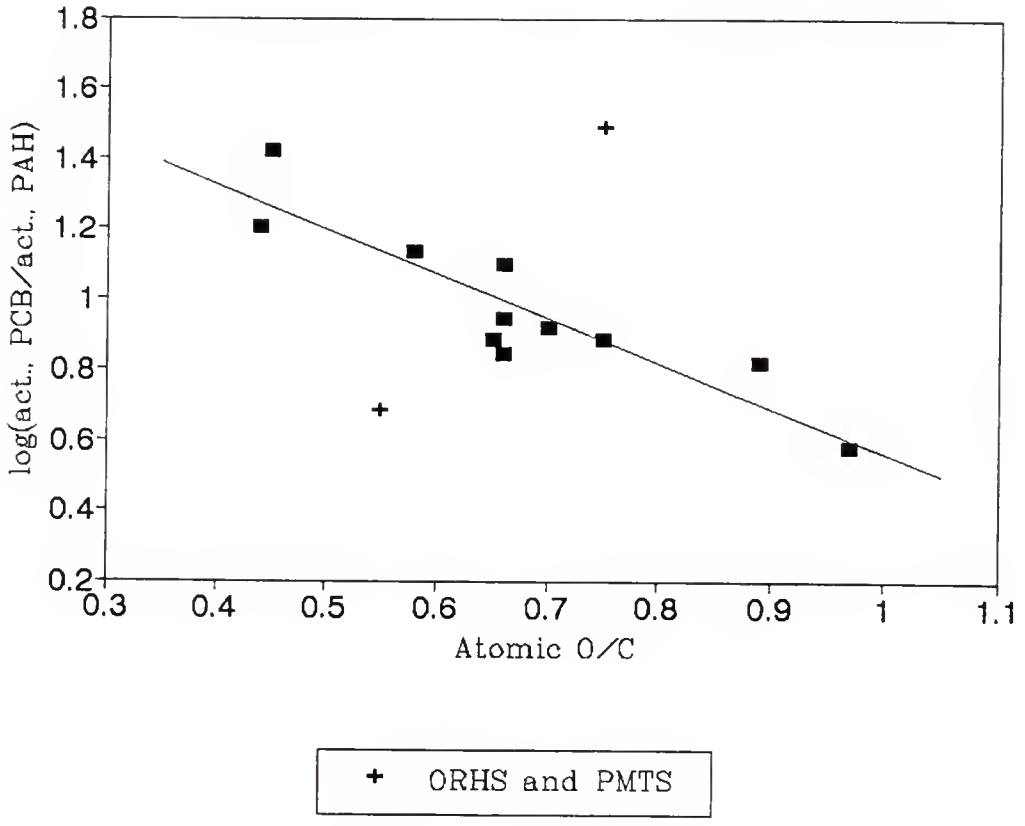

Figure 4.4. An inverse correlation was obtained between the atomic O/C ratio of the humic substances and the $\log(\gamma_{doc,PCB}/\gamma_{doc,PAH})$ ($r^2 = 0.81$, $p < 0.001$). Orange Hts. Soil (ORHTS, upper data point) and Suwannee River Humic Acid (SWRHA, lower data point) were excluded from the correlation calculation.

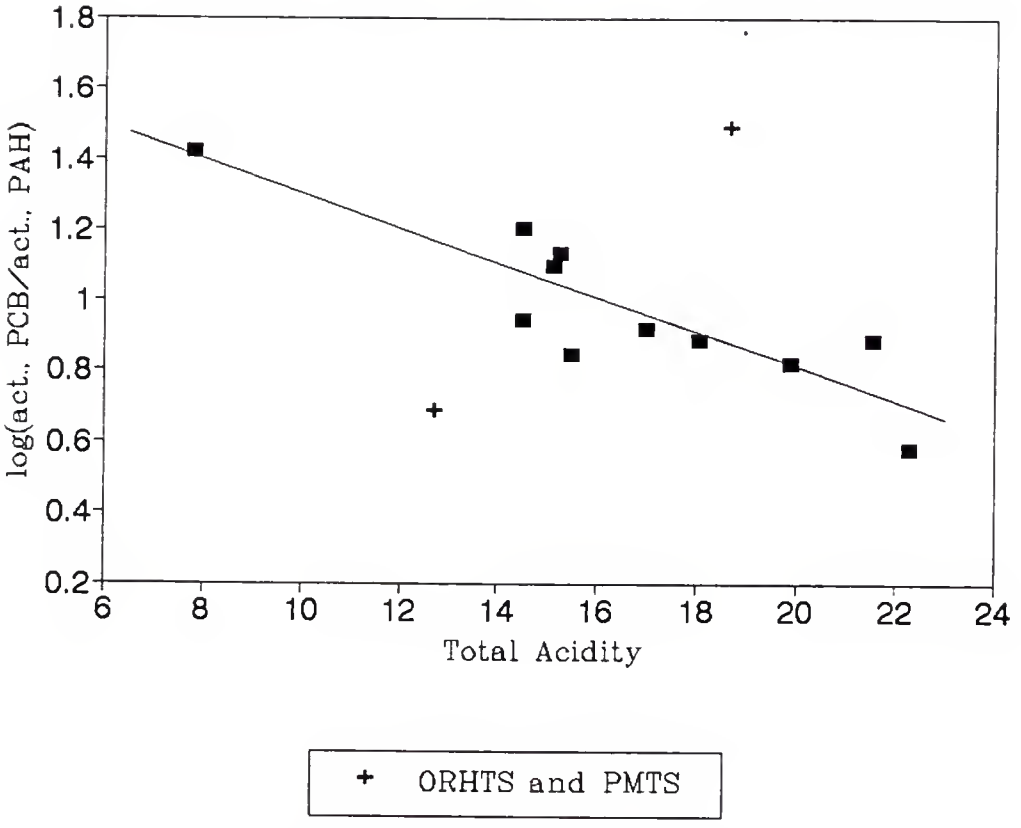

Figure 4.5. An inverse correlation was obtained between the total acidity of the humic substances and the $\log(\gamma_{doc,PCB}/\gamma_{doc,PAH})$ (r^2 = 0.80, $p < 0.001$). Orange Hts. Soil (ORHTS, upper data point) and Suwannee River Humic Acid (SWRHA, lower data point) were excluded from the correlation calculation.

correlations indicate that the K_{doc} decreases as the oxygen content and carboxylic acid content of the humic substances increases. The relationships between $\log(\gamma_{doc,PCB}/\gamma_{doc,PAH})$ and the atomic O/C ratio and the Total Acidity indicate that the variation in the sorption of these two homologous series of HOCs is strongly influenced by the variation in these structural/compositional parameters. Although data from the current study, as well as that available in the literature, indicate that the aromatic carbon content of humic substances is an important characteristic in determining K_{doc}, the role of oxygen containing functional groups must be explored further before an understanding of these interactions can be achieved.

Summary

1. The $\log(K_{doc})$ values measured for the humic substances and HOCs in the current study are within the ranges reported for interactions of HOCs with dissolved humic substances from a variety of sources.

2. Linear relationships were observed between log (S) and $\log(K_{doc})$ for both PCBs and PAHs with each humic substance investigated.

3. Models used to predict the sorption of HOCs from water by soil and sediment organic matter have not been successfully applied to dissolved organic matter due to the

large variation in K_{doc} observed for a particular HOC with different humic substances.

5. The H/C atomic ratio of the humic substances was found to be negatively correlated with the $\log(K_{doc}/K_{ow})$ for both the PCBs ($r^2 = 0.39$, $p < 0.01$) and PAHs ($r^2 = 0.48$, $p < 0.01$).

6. There was a positive correlation observed between the absorptivity at 272 nm for the humic substances and the $\log(K_{doc}/K_{ow})$ for both the PCBs ($r^2 = 0.59$, $p < 0.01$) and PAHs ($r^2 = 0.50$, $p < 0.01$).

7. The IR peak ratio at 1600 cm^{-1} at pH 11 was positively correlated with the $\log(K_{doc}/K_{ow})$ for the PCBs ($r^2 = 0.49$, $p < 0.01$) but not for the PAHs.

8. The aromatic carbon content, measured by ^{13}C NMR, was positively correlated with the $\log(K_{doc}/K_{ow})$ for the PAHs ($r^2 = 0.52$, $p < 0.01$) but not for the PCBs.

9. The $\log(\gamma_{doc,PCB}/\gamma_{doc,PAH})$ was inversely correlated with both the O/C atomic ratio ($r^2 = 0.81$, $p < 0.001$) and the Total Acidity ($r^2 = 0.80$, $p < 0.001$).

Conclusions

1. The results of correlations between log(S) and $\log(K_{doc})$ for the PCBs and PAHs with the humic substances in this dissertation research indicate that the humic substances behave similarly to octanol in that, for all cases, the intercepts of the regression lines for a

particular humic substance are larger for the PAHs than for the PCBs.

2. Based on calculated activity coefficients for each homologous series in the DOC, it can be concluded that the PCBs have higher activities for partitioning into a particular humic substance than do the PAHs.

3. The similarity between the behavior of octanol and that of the dissolved humic substances in this research, relative to their interactions with HOCs, strongly supports a partition mechanism for these interactions.

4. The large variations in the K_{doc} values for a particular HOC with different humic substances observed in this study and reported in the literature indicate that factors other than the hydrophobicity of the HOCs and the carbon content of the humic substances must be considered to explain these interactions.

5. The structure and composition of the humic substances investigated in this study were found to explain some of the variation in $\log(K_{doc})$ values measured for both the PCB and PAH homologous series.

6. The correlations observed between the $\log(K_{doc}/K_{ow})$ and both the atomic H/C ratio and the absorptivity at 272 nm indicate that aromatic and carboxylic acid functional groups of humic substances are important characteristics that influence their interactions with HOCs.

7. The relationships observed for $\log(K_{doc}/K_{ow})$ with the IR peak ratio at 1600 cm^{-1} and with the aromatic carbon content indicate that the PCBs interactions with the humic substances are more sensitive to the oxygen containing functional groups than are the PAHs.

8. The negative slopes of the $\log(\gamma_{doc,PCB}/\gamma_{doc,PAH})$ correlations with the O/C atomic ratio and total acidity indicate that the K_{doc} decreases as the oxygen content and carboxylic acid content of the humic substances increase.

9. The variation in the activity coefficient ratios for the PCBs and PAHs is strongly related to the carboxylic acid content of the humic acids investigated.

10. Data from this dissertation research, as well as those available in the literature, indicate that the aromatic carbon content of humic substances is an important characteristic in determining K_{doc}.

11. The correlations observed in this dissertation research indicate that the oxygen containing functional groups, especially carboxylic acids, of these humic substances are important characteristics which influence their interactions with HOCs.

CHAPTER 5
SUMMARY AND CONCLUSIONS

The sorption of HOCs to natural dissolved organic matter is an important factor in determining the fate of these compounds in aqueous environments. These interactions have been reported to decrease the sorption of HOCs to sediments and suspended particles as well as reduce toxicity and accumulation of HOCs by aquatic biota. Attempts to predict the behavior of HOCs in the presence dissolved humic substances based on previous models of HOC interactions with soil and sediment organic matter have failed. The structure and composition of the dissolved organic matter appear to be more important in these interactions than in those with soil and sediment.

The influence of the structure and composition of dissolved humic substances on their sorption of HOCs was investigated for thirteen humic substances isolated from soil, sedimentary and aqueous environments. A variety of different analytical techniques were employed to elucidate the structure and composition of the humic substances. These methods included elemental analysis, total acidity titration, Cu^{++} binding capacity, UV/Vis spectroscopy,

Fourier transform infrared spectroscopy and ^{13}C nuclear magnetic resonance spectroscopy.

An internal standard infrared spectroscopic method was developed that allowed the quantitative comparison of specific spectral absorption bands between different humic substances. The results of the application of this technique to the humic substances in this dissertation research indicated that a portion of the carboxylic acid functional groups present in these humic substances are strongly hydrogen bonded. These data, in combination with the total acidity and Cu^{++} binding results, suggest that a significant number of the carboxylic acid functional groups in these humic substances are unavailable for metal binding.

The measurement of K_{doc} values for two different homologous series of HOCs with the thirteen different sources of humic substances isolated in this research provided insight into the mechanism of these interactions. The similarity of relationships observed for $\log(K_{ow})$ and $\log(K_{doc})$ with the aqueous solubilities of both the PCBs and PAH support a partition mechanism for the interactions between humic substances and HOCs.

This dissertation research on the relationship between the structure and composition of humic substances and their interactions with HOCs in the dissolved phase is one of only a few such investigations reported to date. The humic substance aromatic carbon content has been proposed to be

the major structural determinant in these interactions. The results of this dissertation research indicate that the aromatic and carboxylic acid functional groups are both important in determining the strength of the interactions between HOCs and dissolved humic substances. Further, the differences in the activities of the PCBs and PAHs for partitioning into the dissolved humic substances indicate that the PCBs are more sensitive to the oxygen containing functional groups of the humic substances than are the PAHs. The inceased sensitivity of PCBs to the oxygen content of the humic substances may be related to the electrophilic nature.

The correlations obtained between the total acidity and both FTIR and ^{13}C NMR spectral data indicate that the oxygenated functional groups of these humic substances are dominated by carboxylic acids. The Cu^{++} binding data for these humic substances indicate that a significant portion of the carboxylic acids are unavailable for metal binding. These findings, in combination with the large portion of carboxylic acids which appear by FTIR to be strongly hydrogen bonded, support a micelle-like model for the conformation of humic substances in solution. Internal hydrogen bonding in humic substances would reduce the polarity of oxygen containing functional groups within the humic molecular structure. This decrease in polarity within

the humic molecular structure could cause an increase in HOC sorption.

Humic substances are a diverse mixture of complex macromolecules. Investigation of any characteristic of humic substances is hampered by the variation introduced by their very nature. However, the application of multiple analytical methods to an investigation of the structure, composition and reactions of humic substances has yielded some understanding of these important natural organic compounds. The uncertainty inherent in the investigation of humic substances requires that large databases be used before meaningful general conclusions can be drawn about any particular characteristic.

Attempts to extend models developed for the sorption of HOCs by soil and sediment organic matter to dissolved humic substances have been unsuccessful because they only consider the quantity of organic carbon present in soils and sediment. The research presented in this dissertation confirms that the qualitative aspects (i.e. structure and composition) of dissolved humic substances are important factors which influence their interactions with HOCs.

Future Research

The results presented in this dissertation add to the relatively small amount of information currently available on the relationships between the structure and composition

of humic substances and their sorption of HOCs. The complex nature of humic substances requires large data sets for statistically significant conclusions to be obtained about any aspect of their behavior. New analytical techniques for the structural investigation of complex organic molecules, especially FTIR, ^{13}C NMR, and tandem mass spectrometry, will be important tools for continued research on humic substance structure and composition. The application of these techniques to additional sources of humic substances will be required before a general understanding and predictive model of the interactions between dissolved humic substances and hydrophobic organic compounds can be attained.

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APPENDIX A

ADDITIONAL DATA ON THE CHARACTERIZATION OF HUMIC SUBSTANCE STRUCTURE AND COMPOSITION

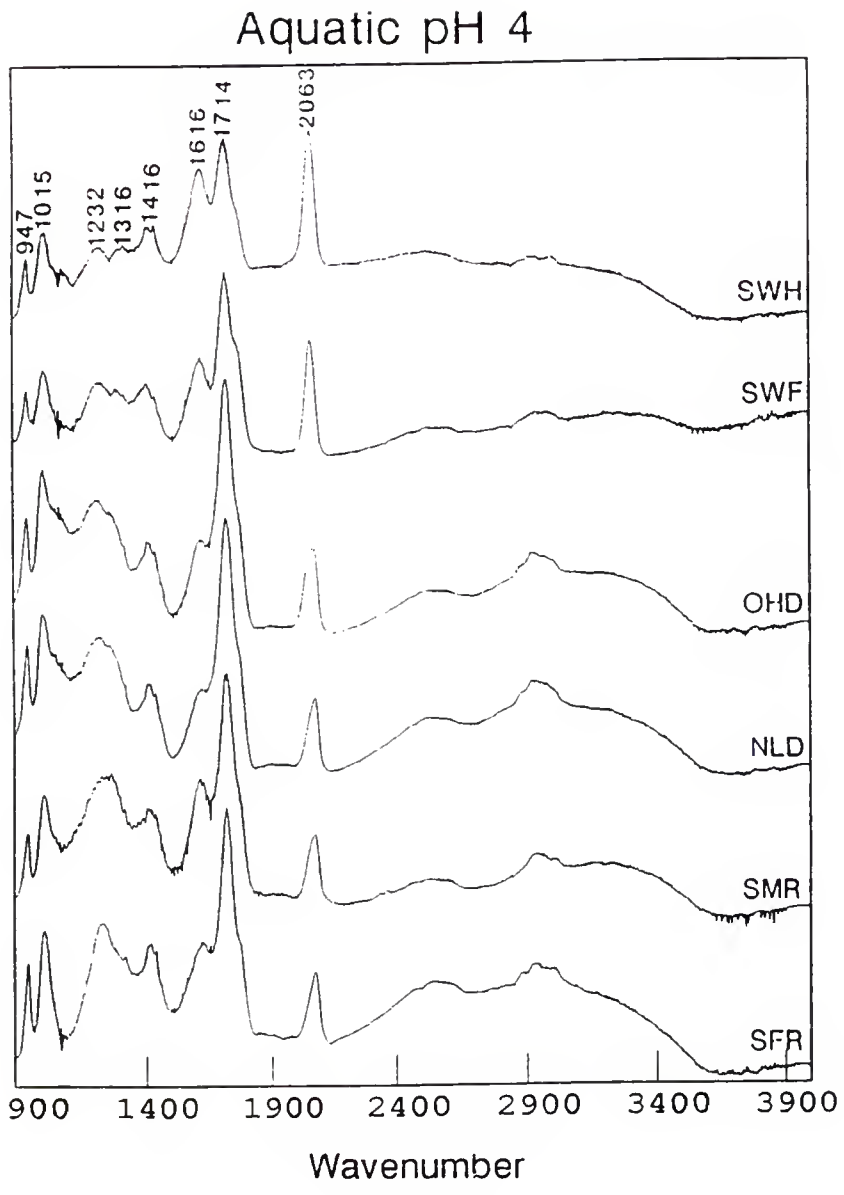

Figure A1.1 Fourier transform infrared spectra recorded at pH 4 in 50% DMSO for the aquatic humic substances. Abreviations are as follows: SFR = Santa Fe River; SMR = St. Marys River; NLD = Newnans Lake DOC; OHD = Orange Hts. DOC; SWF = Suwannee River Fulvic Acid; SWH = Suwannee River Humic Acid. The vertical axis represents absorbance.

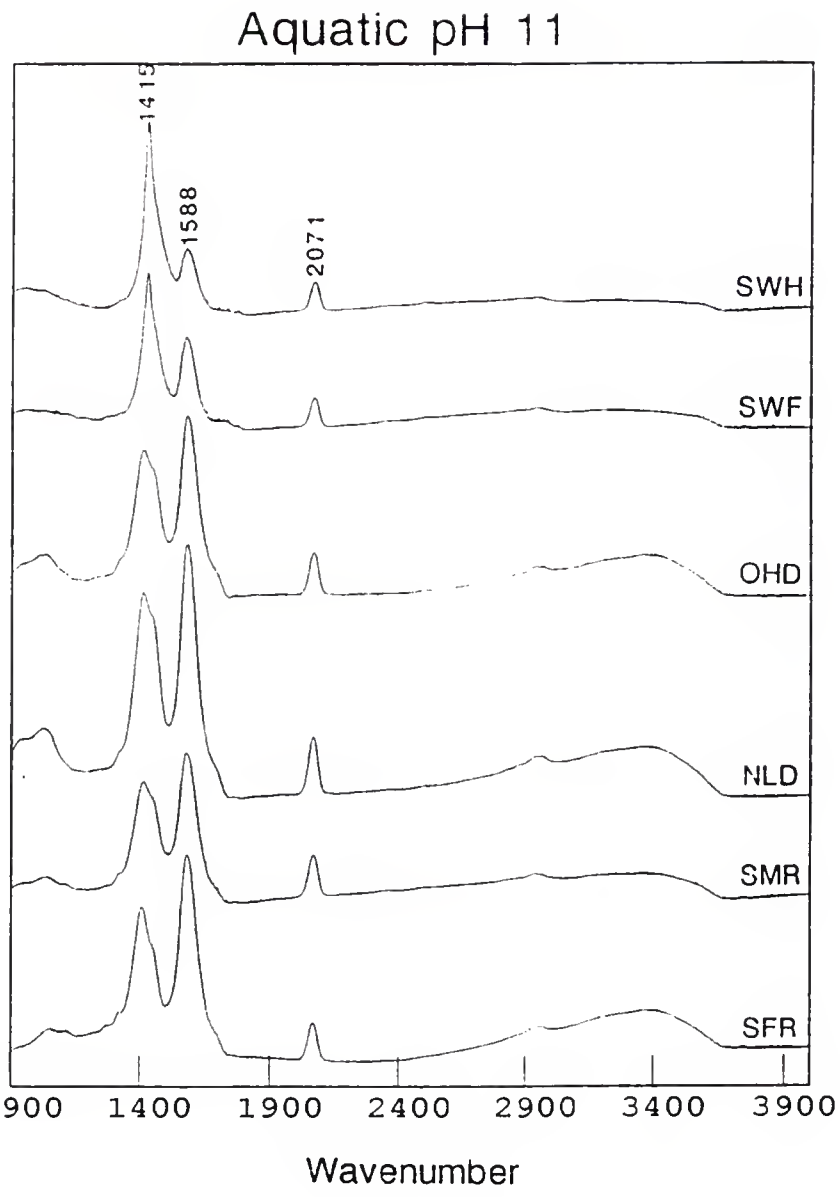

Figure A1.2 Fourier transform infrared spectra recorded at pH 11 in deionized water for the aquatic humic substances. Abreviations are as follows: SFR = Santa Fe River; SMR = St. Marys River; NLD = Newnans Lake DOC; OHD = Orange Hts. DOC; SWF = Suwannee River Fulvic Acid; SWH = Suwannee River Humic Acid. The vertical axis represents absorbance.

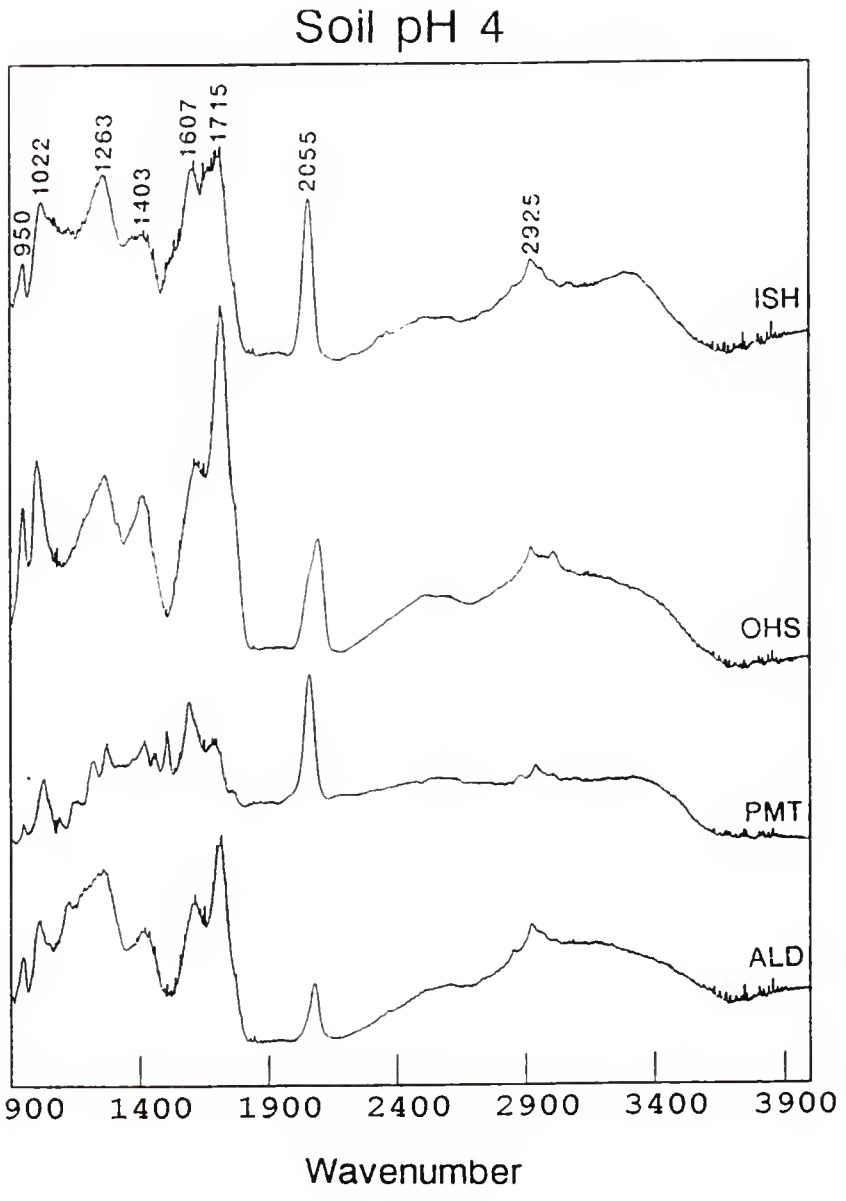

Figure A1.3 Fourier transform infrared spectra recorded at pH 4 in 50% DMSO for the soil humic substances. Abreviations are as follows: ALD = Aldrich Humic Acid; PMT = Pime Mt.; OHS = Orange Hts. Soil; ISH = International Humic Substances Society Soil. The vertical axis represents absorbance.

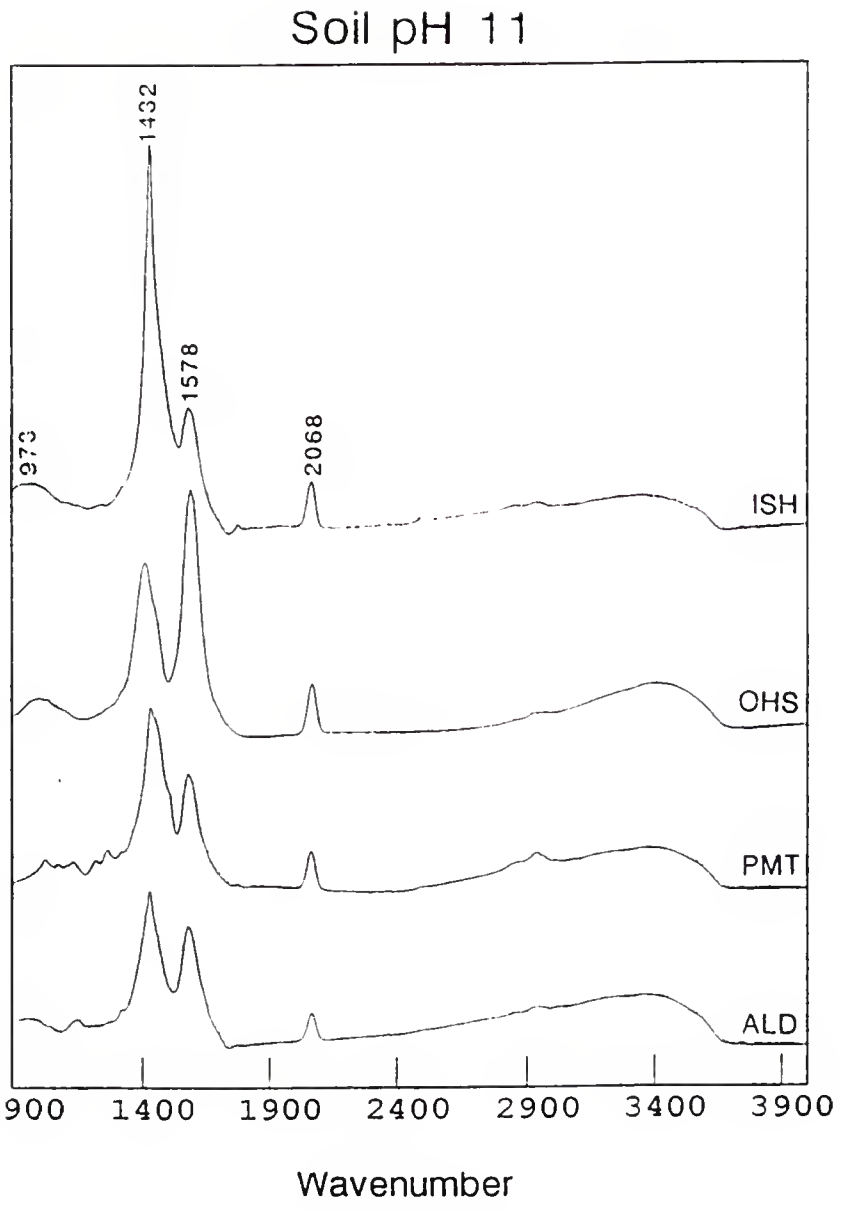

Figure A1.4 Fourier transform infrared spectra recorded at pH 11 in deionized water for the soil humic substances. Abreviations are as follows: ALD = Aldrich Humic Acid; PMT = Pime Mt.; OHS = Orange Hts. Soil; ISH = International Humic Substances Society Soil. The vertical axis represents absorbance.

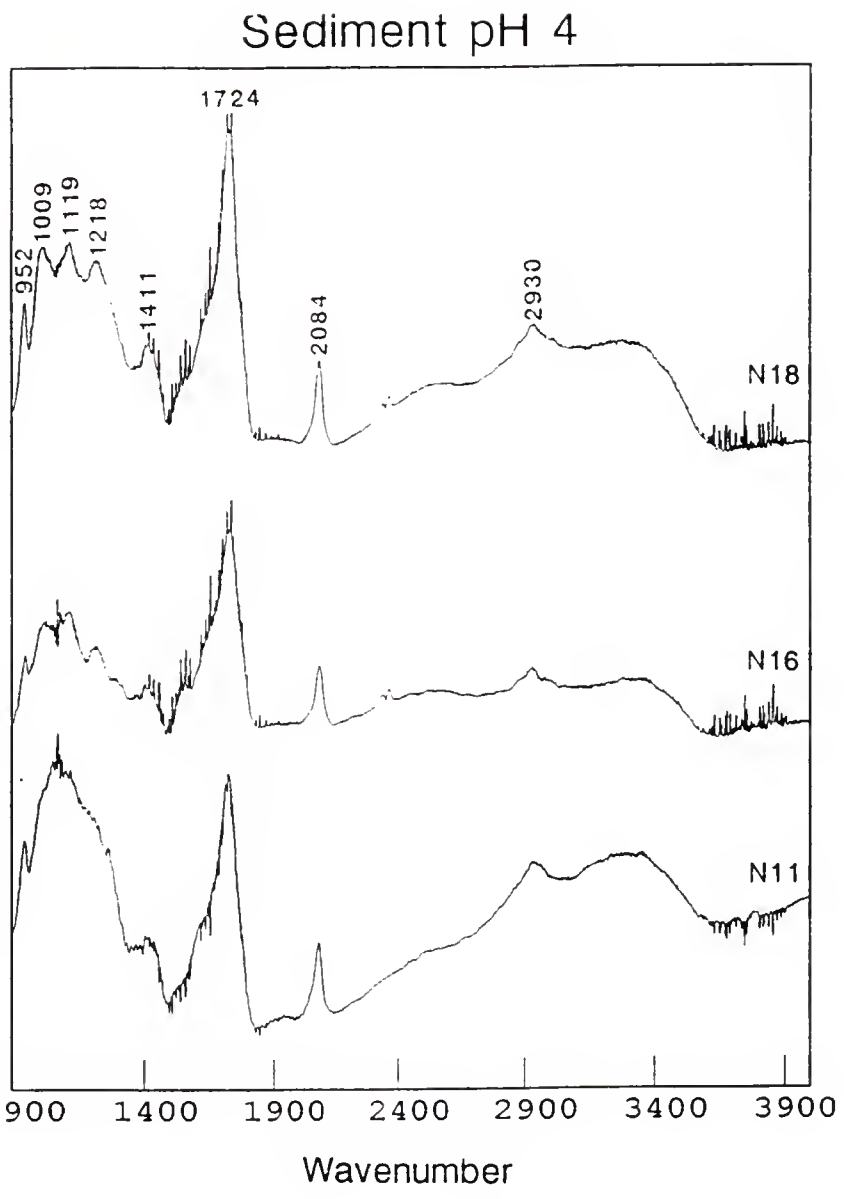

Figure A1.5 Fourier transform infrared spectra recorded at pH 4 in 50% DMSO for the sediment humic substances. Abreviations are as follows: N11= Newnans Lake Sediment 11; N16= Newnans Lake Sediment 16; N18= Newnans Lake Sediment 18. The vertical axis represents absorbance.

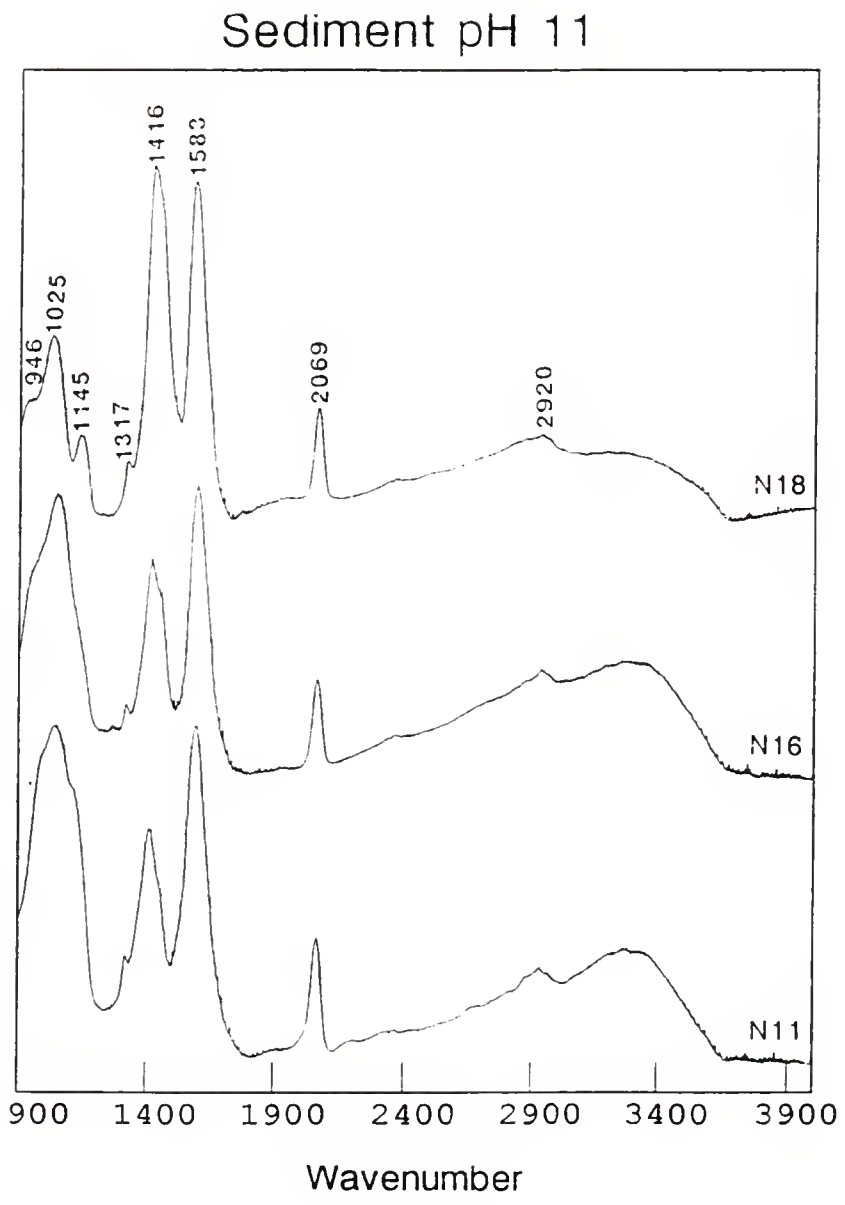

Figure A1.6 Fourier transform infrared spectra recorded at pH 11 in deionized water for the sediment humic substances. Abreviations are as follows: N11= Newnans Lake Sediment 11; N16= Newnans Lake Sediment 16; N18= Newnans Lake Sediment 18. The vertical axis represents absorbance.

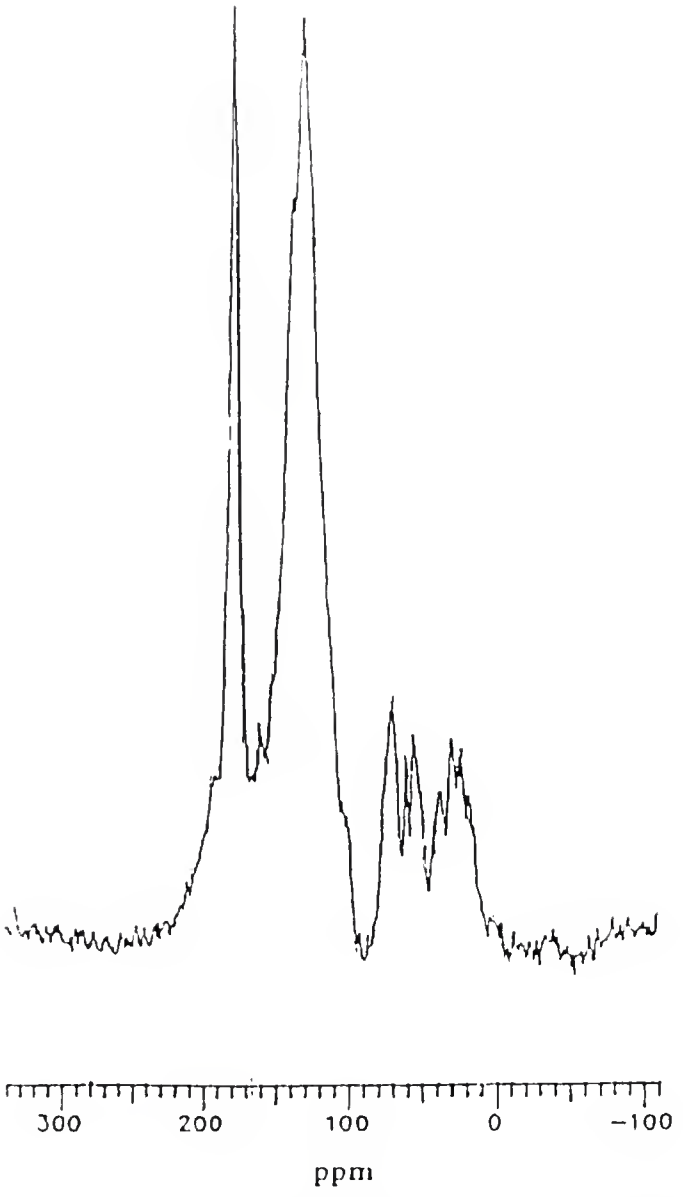

Figure A1.7 ^{13}C nuclear magnetic resonance spectra recorded for IHSS Humic Acid. Spectra recorded from dimethylsulfoxide solution. Chemical shift is reported in parts per million relative to tetramethylsilane.

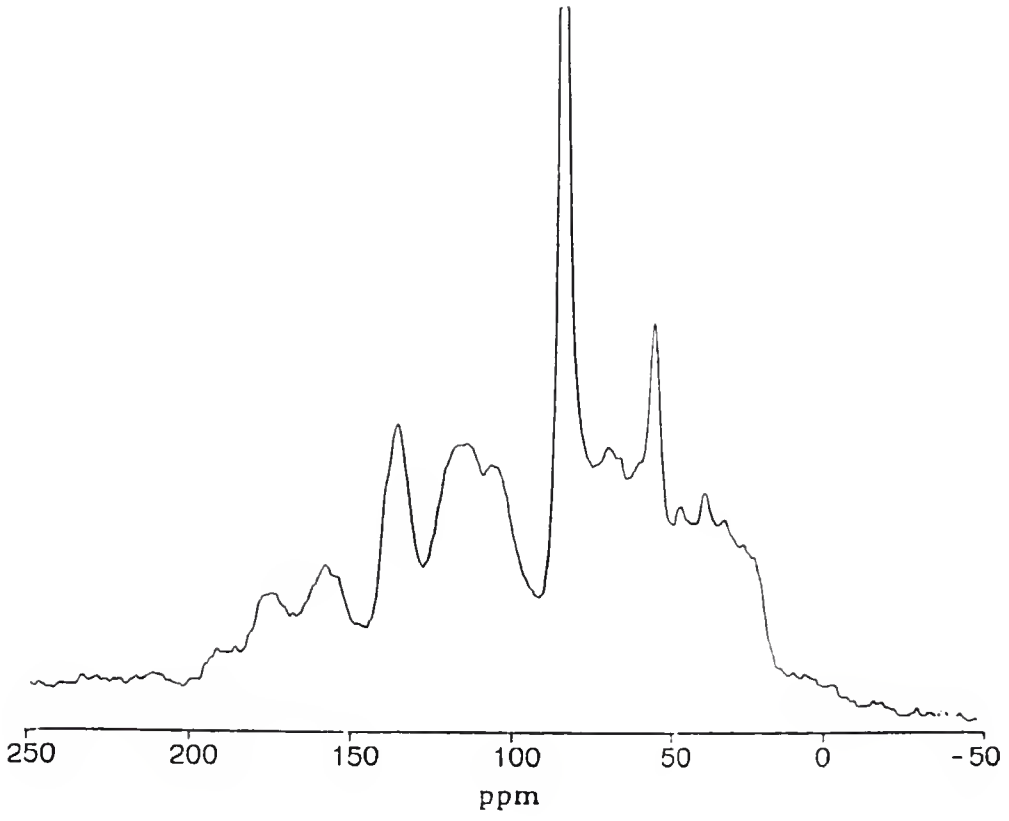

Figure A1.8 ^{13}C nuclear magnetic resonance spectra recorded for Pine Mt. Soil. Spectra recorded from solid sample. Chemical shift is reported in parts per million relative to tetramethylsilane. The peak at 85 ppm is the Delrin internal standard.

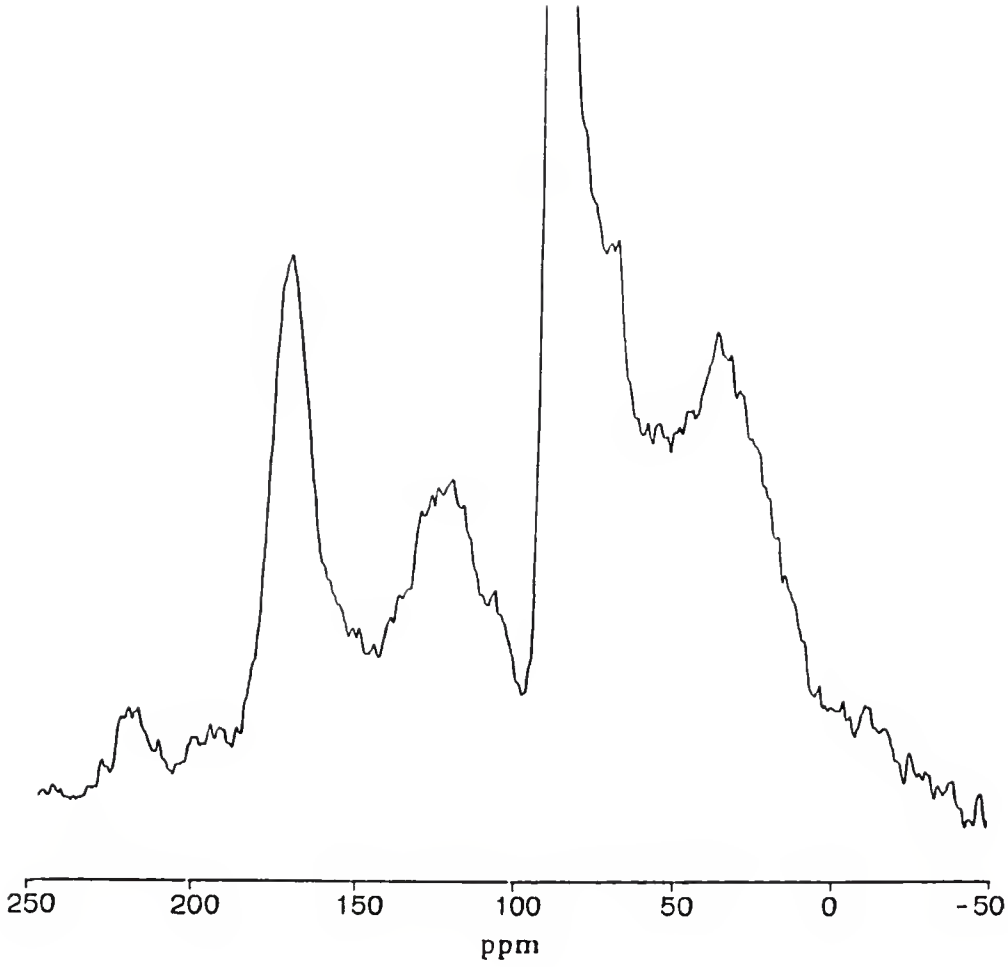

Figure A1.9 ^{13}C nuclear magnetic resonance spectra recorded for Orange Hts. Soil. Spectra recorded from solid sample. Chemical shift is reported in parts per million relative to tetramethylsilane. The peak at 85 ppm is the Delrin internal standard.

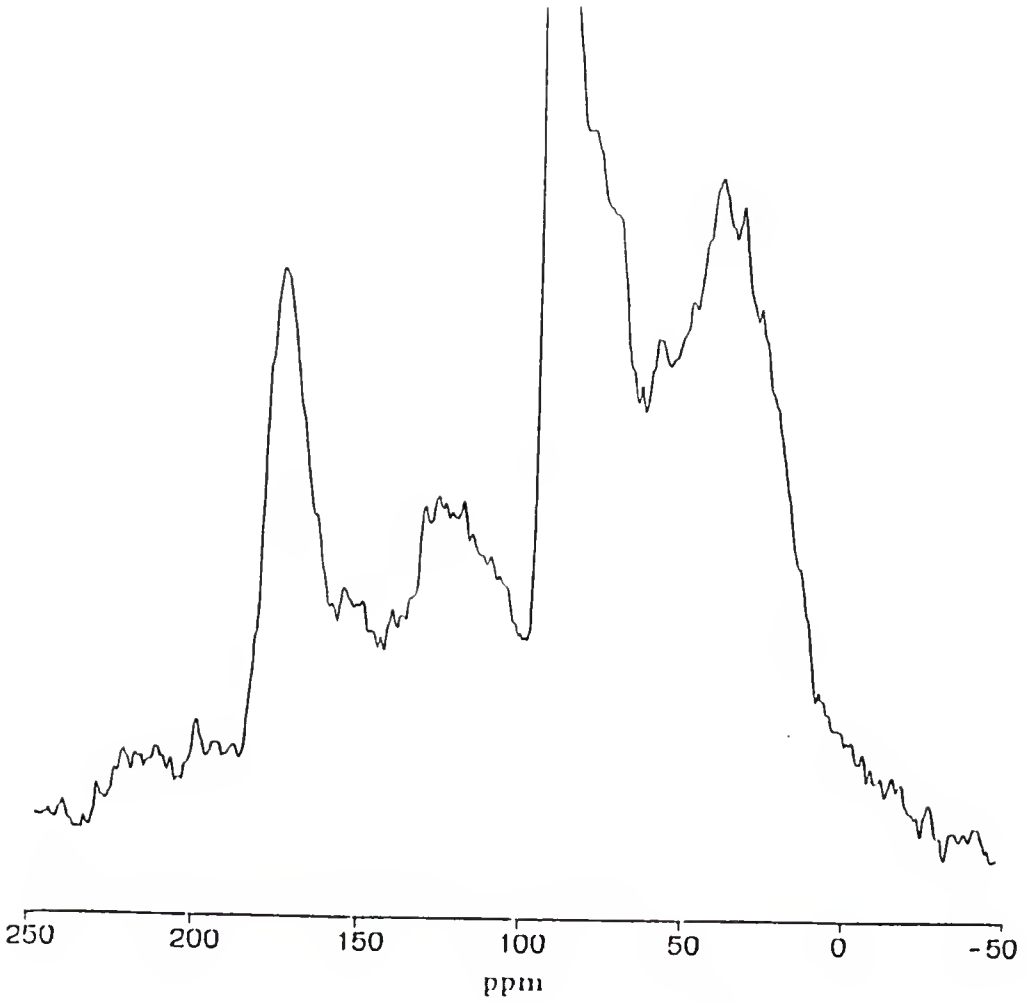

Figure A1.10 ^{13}C nuclear magnetic resonance spectra recorded for Santa Fe River DOC. Spectra recorded from solid sample. Chemical shift is reported in parts per million relative to tetramethylsilane. The peak at 85 ppm is the Delrin internal standard.

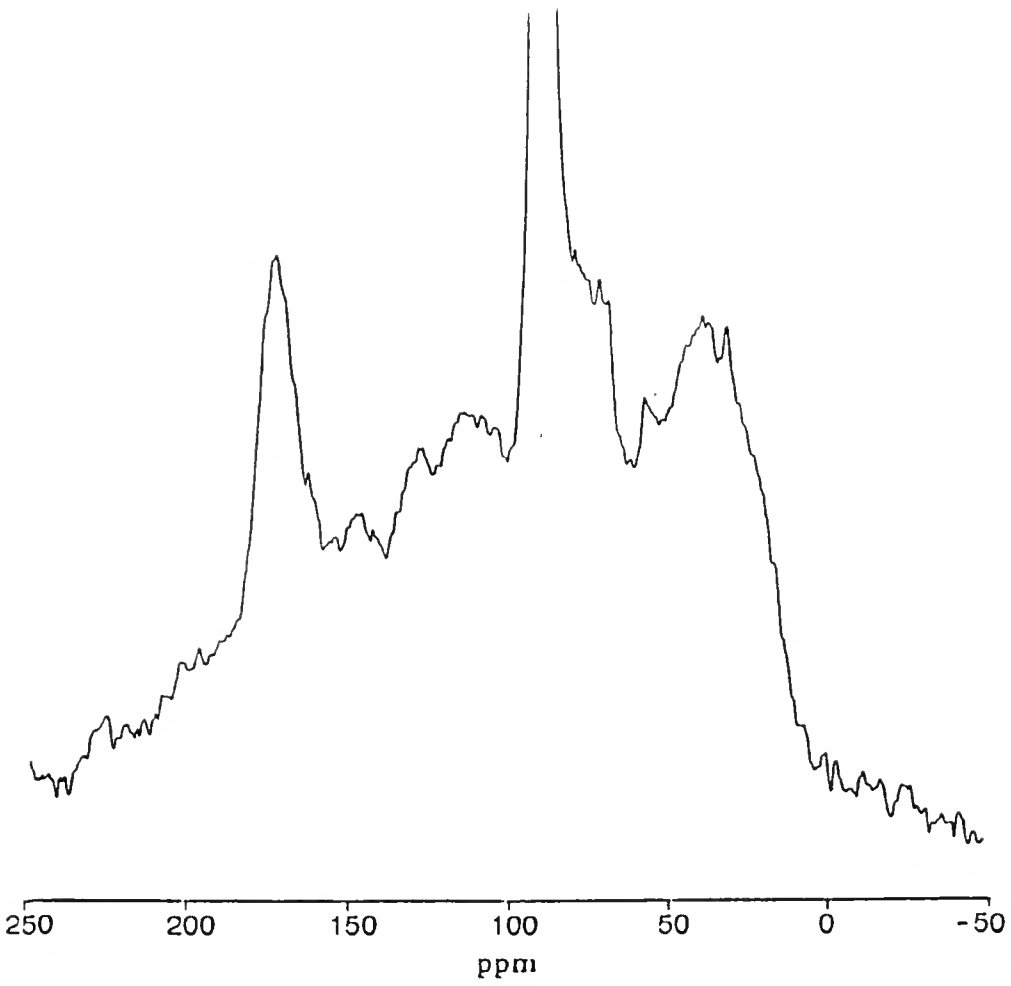

Figure A1.11 ^{13}C nuclear magnetic resonance spectra recorded for St. Marys River DOC. Spectra recorded from solid sample. Chemical shift is reported in parts per million relative to tetramethylsilane. The peak at 85 ppm is the Delrin internal standard.

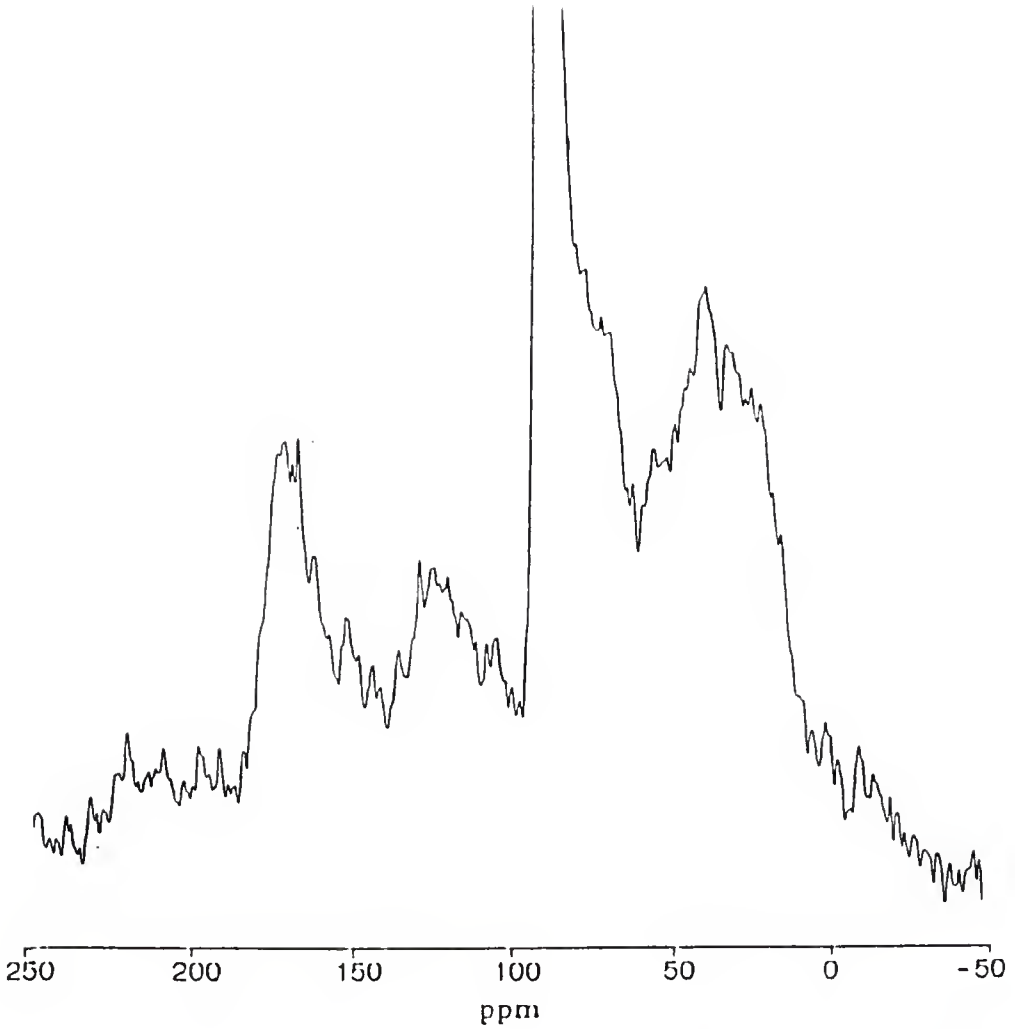

Figure A1.12 ^{13}C nuclear magnetic resonance spectra recorded for Orange Hts. DOC. Spectra recorded from solid sample. Chemical shift is reported in parts per million relative to tetramethylsilane. The peak at 85 ppm is the Delrin internal standard.

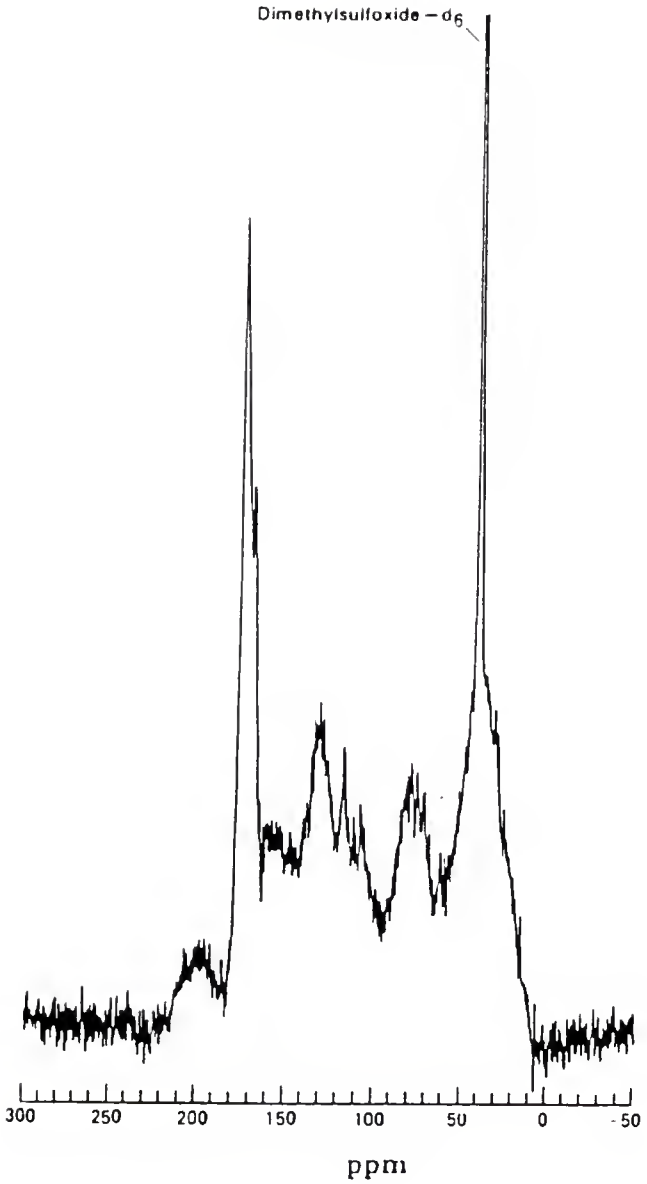

Figure A1.13 ^{13}C nuclear magnetic resonance spectra recorded for Suwannee River Fulvic Acid. Spectra recorded from dimethylsulfoxide solution. Chemical shift is reported in parts per million relative to tetramethylsilane.

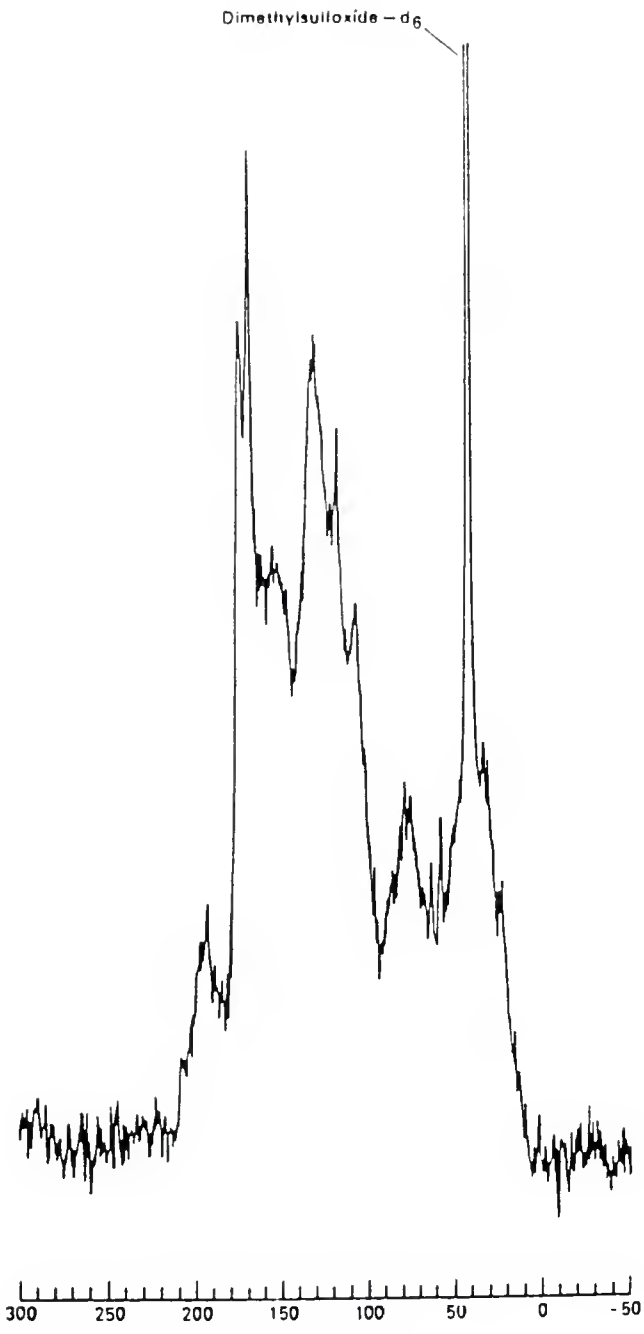

Figure A1.14 ^{13}C nuclear magnetic resonance spectra recorded for Suwannee River Humic Acid. Spectra recorded from dimethylsulfoxide solution. Chemical shift is reported in parts per million relative to tetramethylsilane.

APPENDIX B

ADDITIONAL DATA ON THE INTERACTIONS OF HYDROPHOBIC ORGANIC COMPOUNDS WITH DISSOLVED HUMIC SUBSTANCES

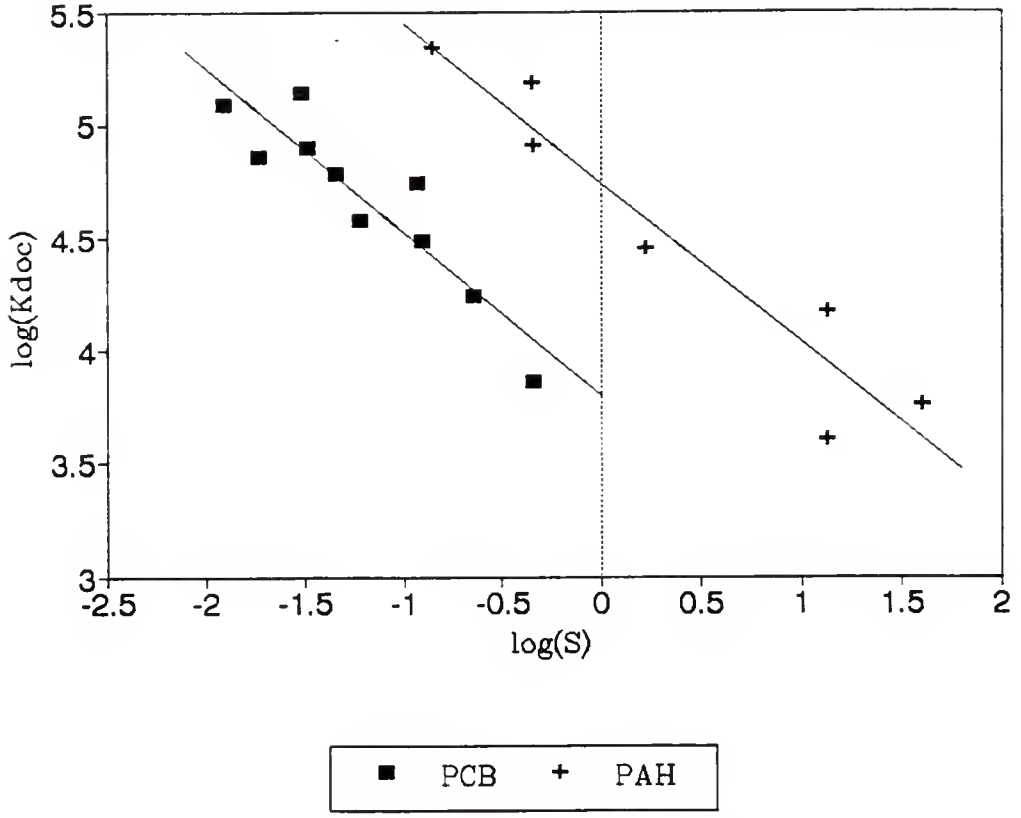

Figure B1.1 The log(Sol.) was correlated with the log(K_{doc}) for Aldrich Humic Acid and both the PCBs and PAHs. The slopes are not significantly different, statistically, while the intercepts are significantly different (see Table 4.4).

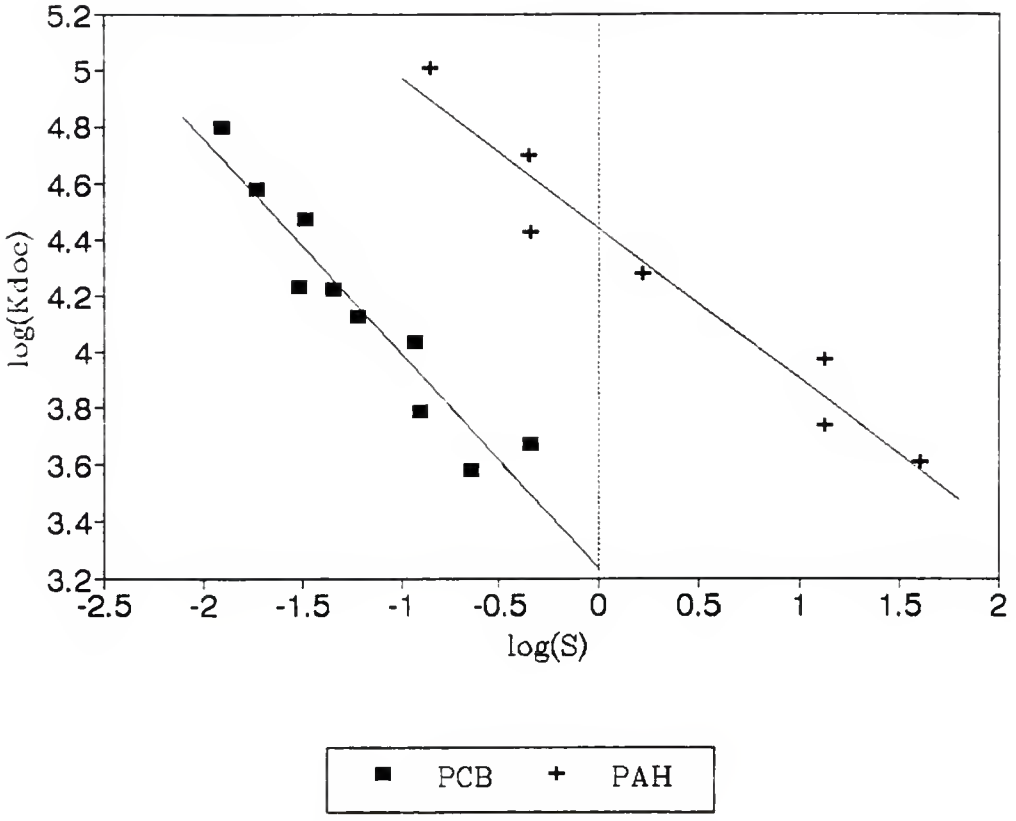

Figure B1.2 The log(Sol.) was correlated with the log(K_{doc}) for IHSS Humic Acid and both the PCBs and PAHs. The slopes are not significantly different, statistically, while the intercepts are significantly different (see Table 4.4).

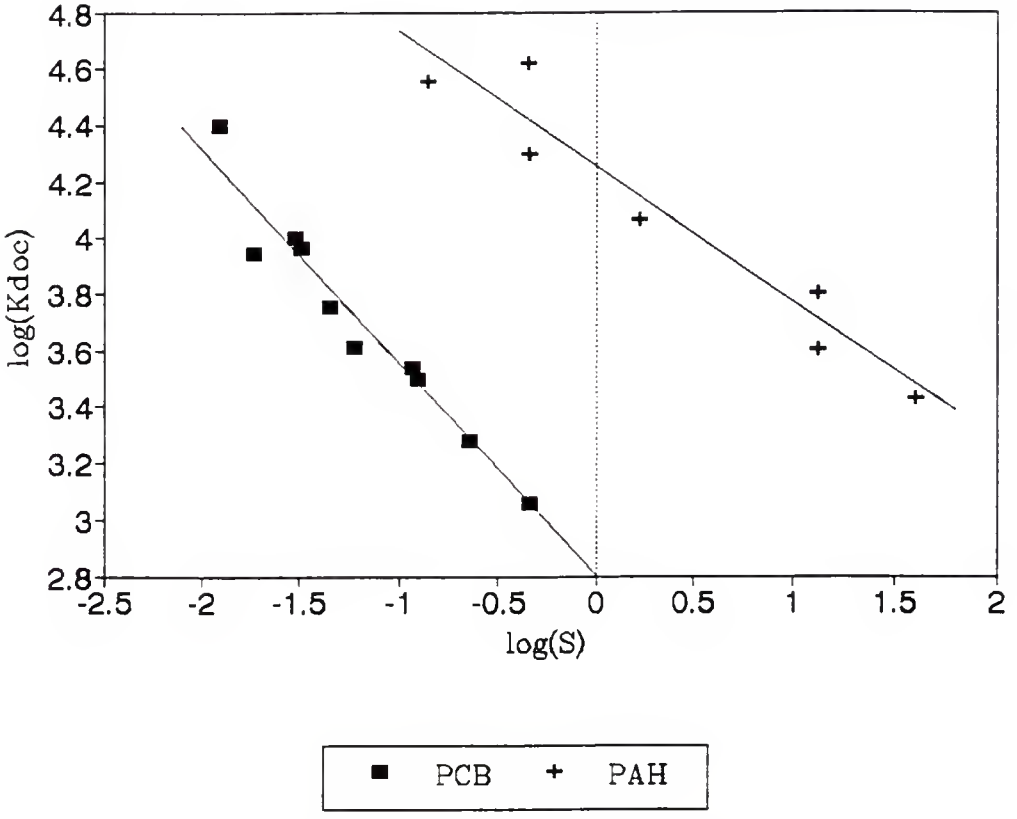

Figure B1.3 The log(Sol.) was correlated with the log(K_{doc}) for Pine Mt. Soil and both the PCBs and PAHs. The slopes are not significantly different, statistically, while the intercepts are significantly different (see Table 4.4).

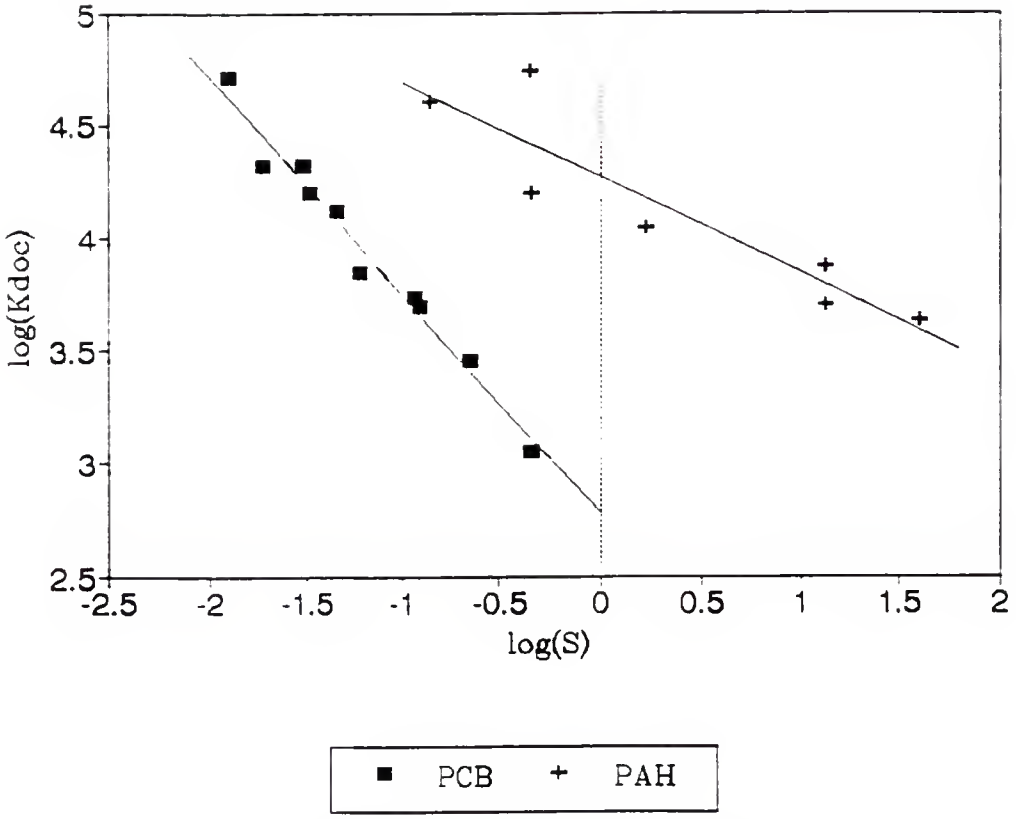

Figure B1.4 The log(Sol.) was correlated with the log(K_{doc}) for Orange Hts. Soil and both the PCBs and PAHs. The slopes are not significantly different, statistically, while the intercepts are significantly different (see Table 4.4).

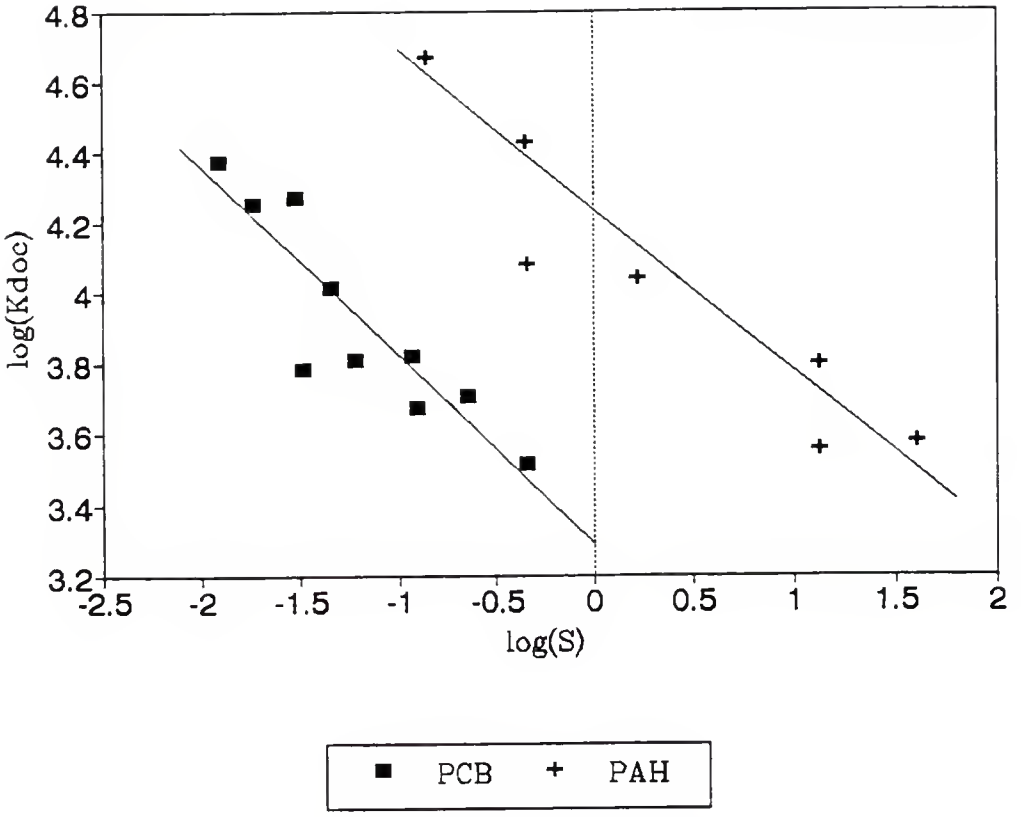

Figure B1.5 The log(Sol.) was correlated with the log(K_{doc}) for Newnans Lake Sediment 11 and both the PCBs and PAHs. The slopes are not significantly different, statistically, while the intercepts are significantly different (see Table 4.4).

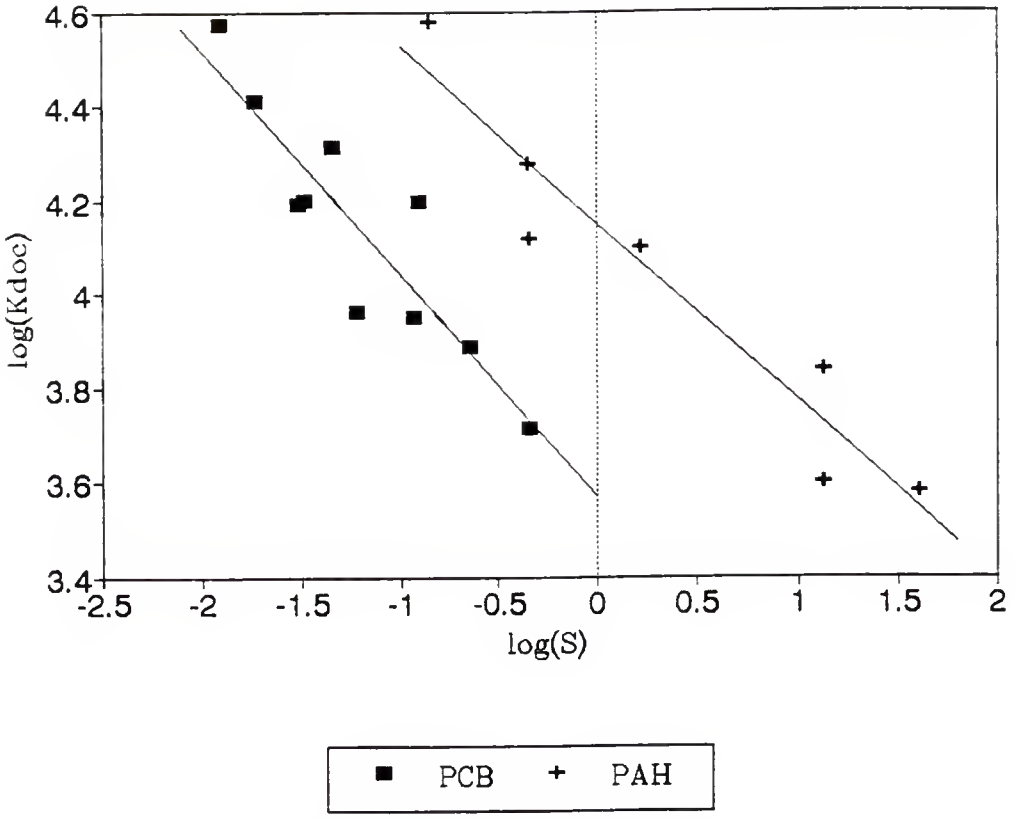

Figure B1.6 The log(Sol.) was correlated with the log(K_{doc}) for Newnans Lake Sediment 16 and both the PCBs and PAHs. The slopes are not significantly different, statistically, while the intercepts are significantly different (see Table 4.4).

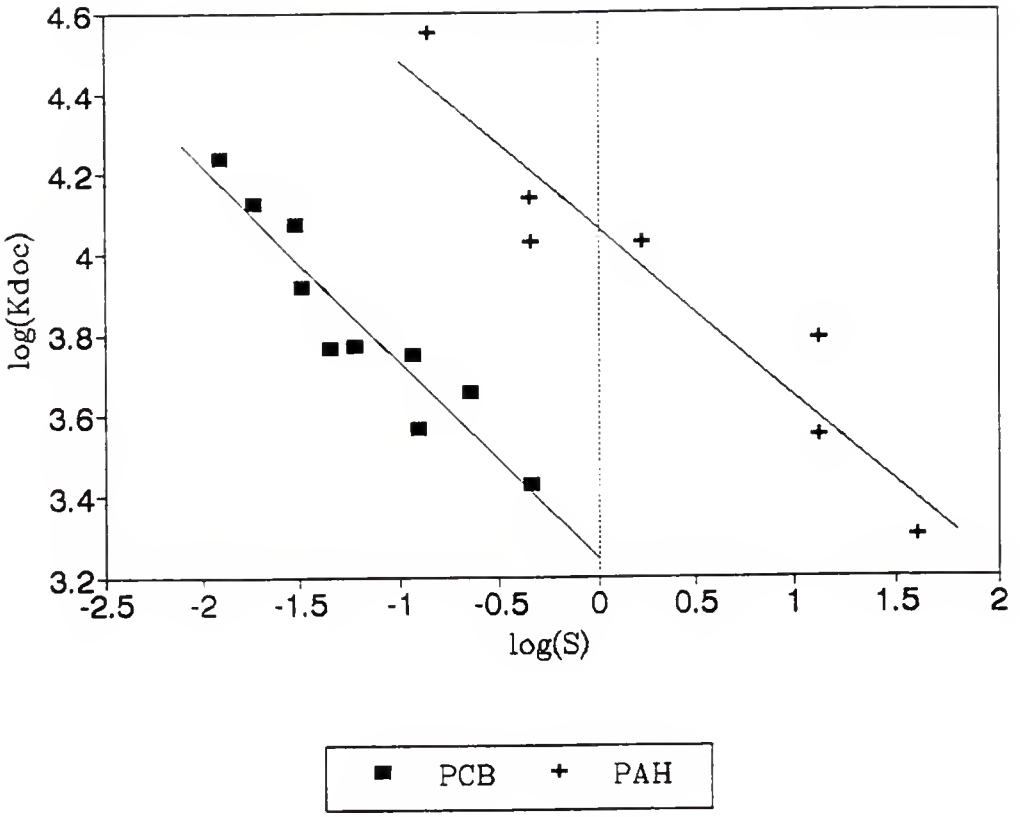

Figure B1.7 The log(Sol.) was correlated with the log(K_{doc}) for Newnans Lake Sediment 18 and both the PCBs and PAHs. The slopes are not significantly different, statistically, while the intercepts are significantly different (see Table 4.4).

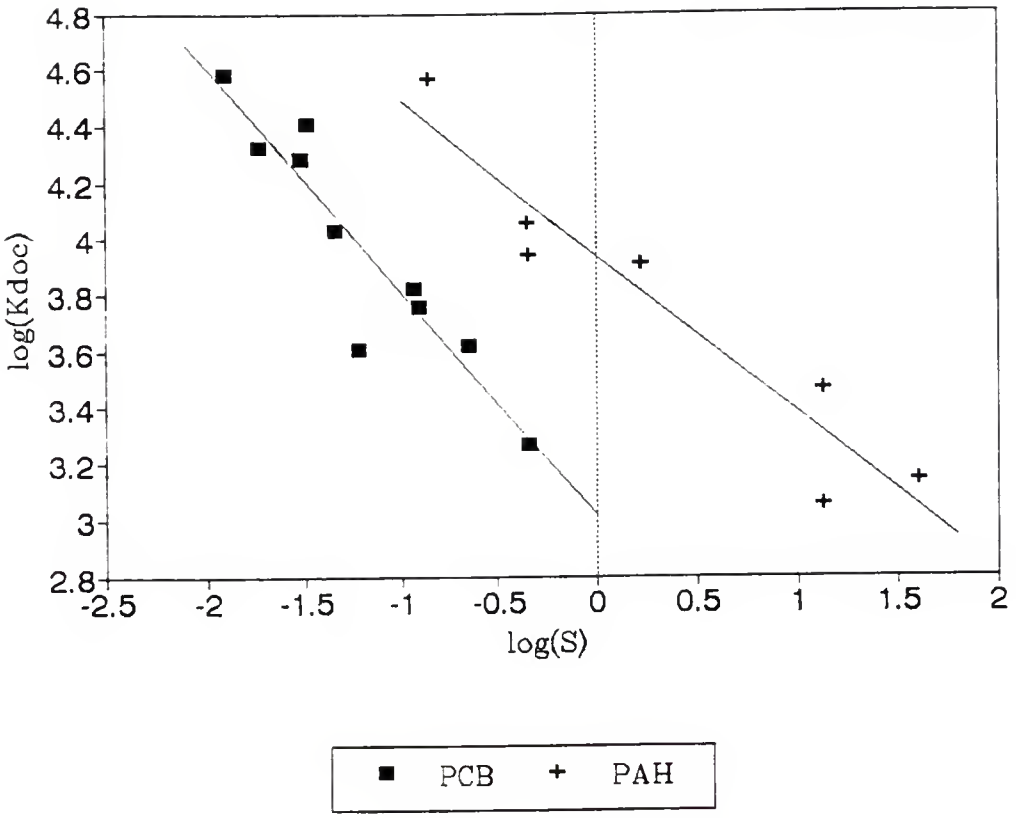

Figure B1.8 The log(Sol.) was correlated with the log(K_{doc}) for Santa Fe River DOC and both the PCBs and PAHs. The slopes are not significantly different, statistically, while the intercepts are significantly different (see Table 4.4).

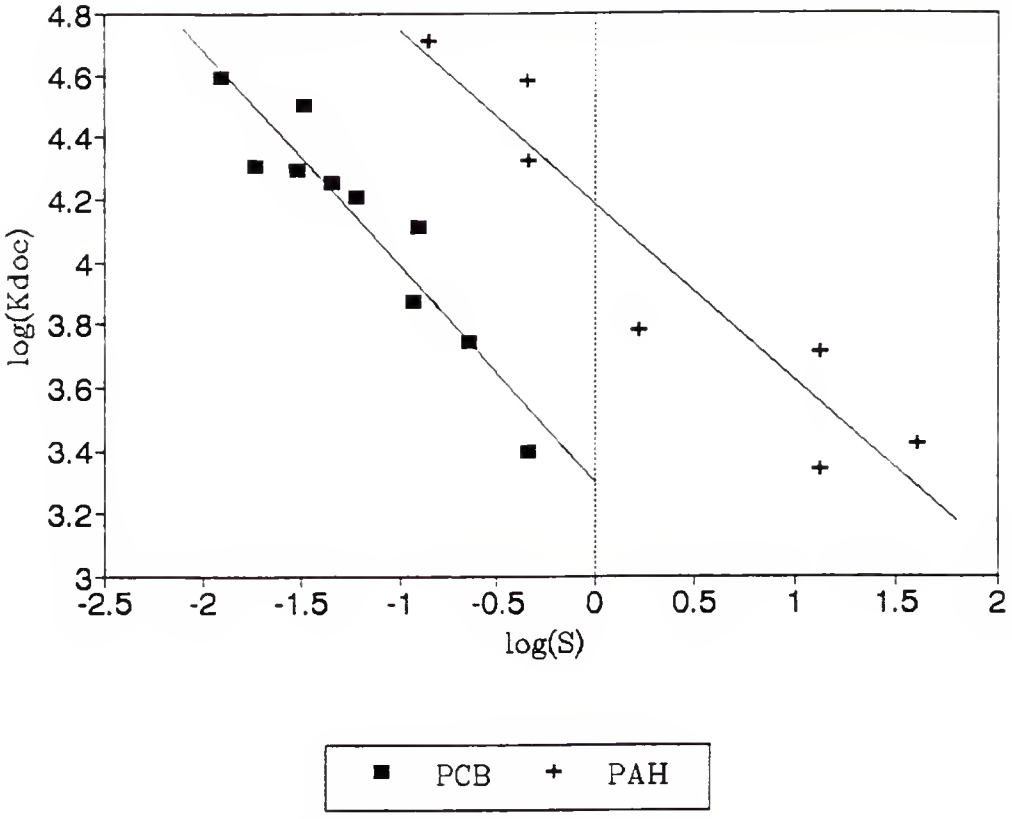

Figure B1.9 The log(Sol.) was correlated with the log(K_{doc}) for St. Marys River DOC and both the PCBs and PAHs. The slopes are not significantly different, statistically, while the intercepts are significantly different (see Table 4.4).

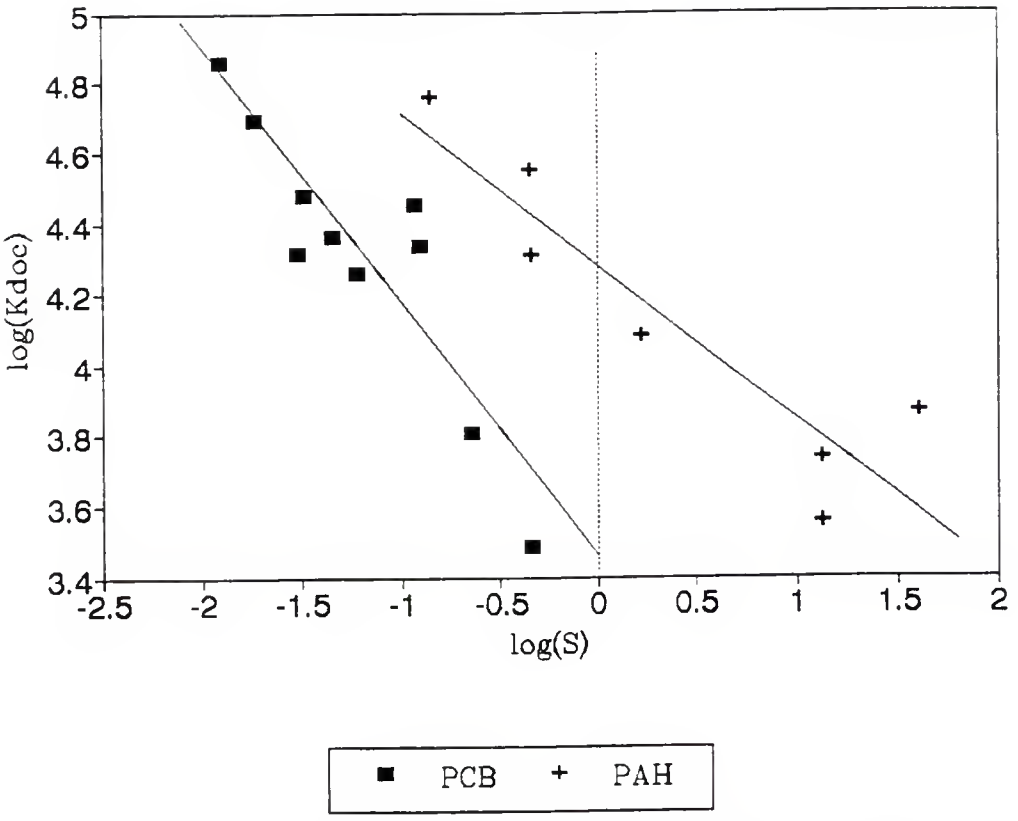

Figure B1.10 The log(Sol.) was correlated with the log(K_{doc}) for Orange Hts. DOC and both the PCBs and PAHs. The slopes are not significantly different, statistically, while the intercepts are significantly different (see Table 4.4).

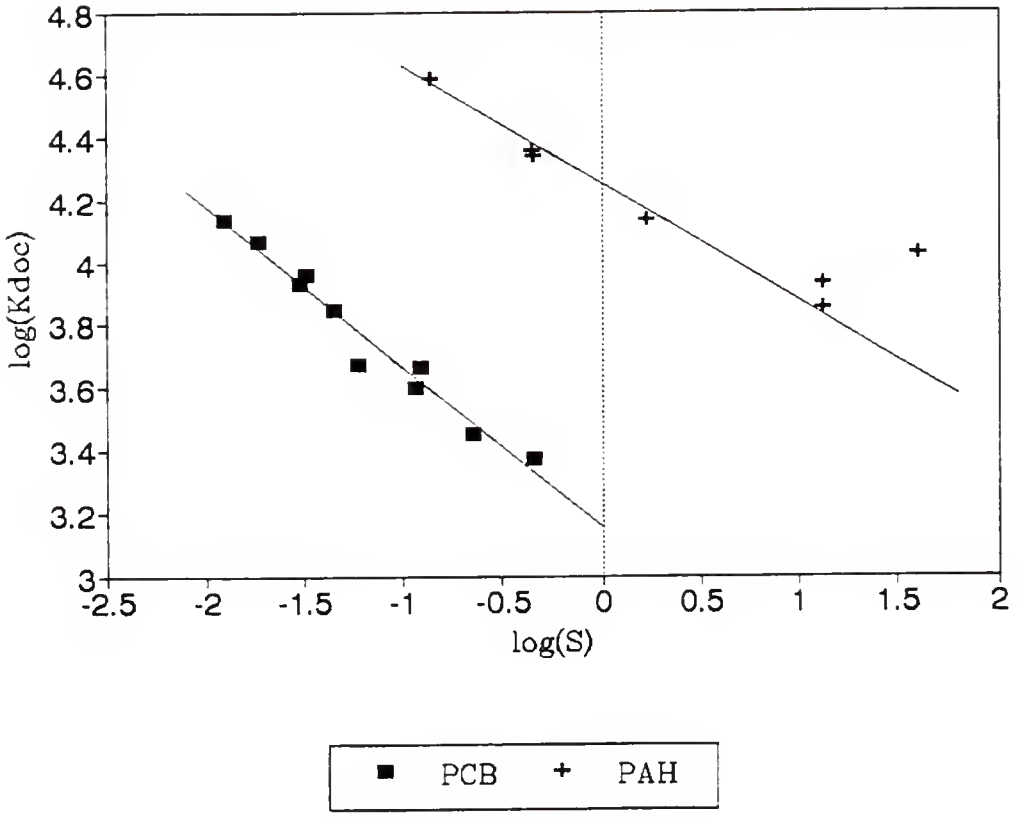

Figure B1.11 The log(Sol.) was correlated with the log(K_{doc}) for Newnans Lake DOC and both the PCBs and PAHs. The slopes are not significantly different, statistically, while the intercepts are significantly different (see Table 4.4).

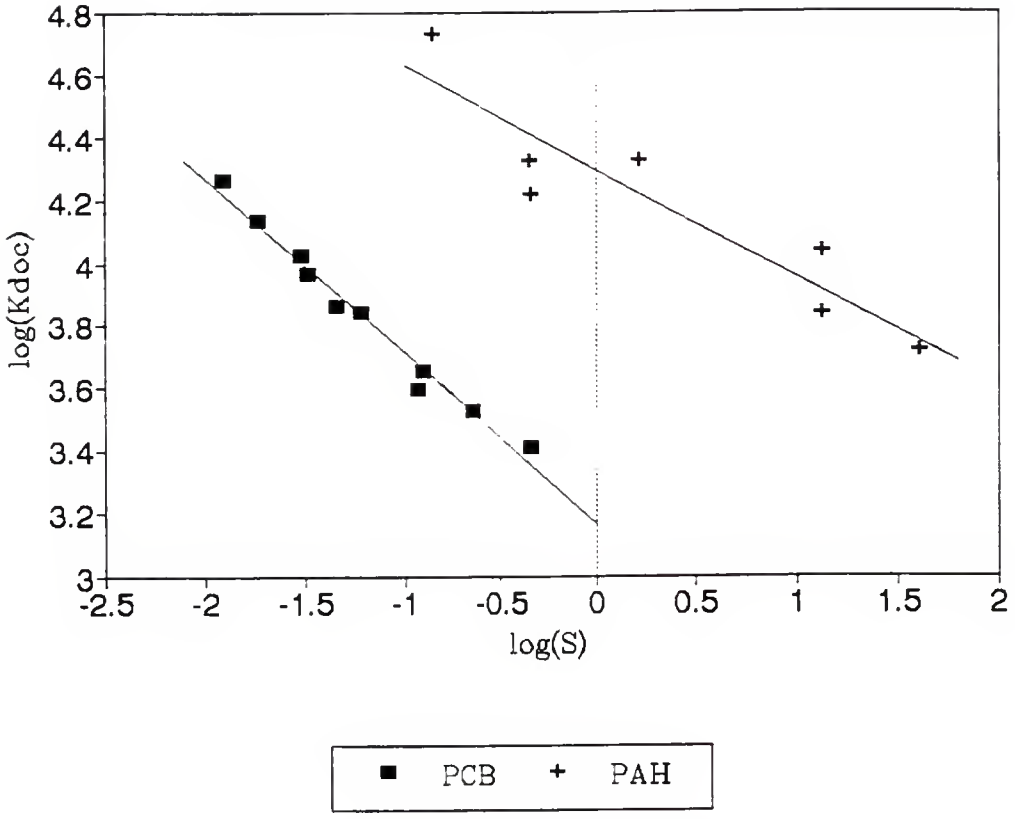

Figure B1.12 The log(Sol.) was correlated with the log(K_{doc}) for Suwannee River Fulvic Acid and both the PCBs and PAHs. The slopes are not significantly different, statistically, while the intercepts are significantly different (see Table 4.4).

BIOGRAPHICAL SKETCH

William McCord Davis was born in Philadelphia, Pennsylvania, on December 19, 1953. He grew up in northern Alabama and east/central Florida. He received a bachelor of science degree in Chemistry from Florida State University in 1976. He received a master of science degree in Analytical Chemistry from Florida State University in 1985. After working in environmental analytical chemistry for a number of years, he returned to graduate school to obtain a Doctor of Philosophy degree from the University of Florida. He intends to pursue a career in teaching at the university level.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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